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THE CHEMISTRY OF THE ANAEROBIC MUSCULAR CONTRACTION[1]

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First of all, I wish to express my most sincere thanks to the Dohme Lectureship Committee for the great honour shown me by their invitation to deliver the Dohme lectures of this year.

I imagine there must have been some connection between this great honour, which I highly appreciate, and that piece of good luck which I had seven or eight years ago, when I started my experiments on the effect of iodo-acetic acid on the chemistry of muscular contraction. For that reason I have chosen the chemistry of anaerobic muscular contraction as the subject of my first lecture. I have chosen this subject for the purpose of getting an opportunity to give a kind of review of the development of the chemistry of muscular contraction during the years previous to and following 1929, when I started my first experiments in this field.

In 1929 the discussion between the school of Embden and the school of Meyerhof was going on with unabated vigour. The subject of the discussion was, mainly, the rôle played by the formation of lactic acid in the chemistry of muscular contraction. Embden denied that formation of lactic acid could possibly play any part in what we may call the mechanism of muscular contraction. By this term I understand the physico-chemical changes which are the immediate cause of the mechanical response of the muscle, *i.e.* the shortening or development of tension. Meyerhof, on his side, claimed that the formation of lactic acid is an absolutely necessary link in the chemistry of muscu-

[1] Lecture I of the thirteenth series of lectures under The Charles E. Dohme Memorial Lectureship of The Johns Hopkins University School of Medicine. Delivered November 10, 1937. Lecture II, *The Metabolism of the Aerobic Working Muscles*, appears in this same number of the BULLETIN; Lecture III, *The Metabolism of the Isolated Liver*, will appear in a subsequent issue.

lar contraction, closely connected with the mechanism of contraction. Moreover, according to Meyerhof, the formation of lactic acid was the main source and probably even the only source of energy during anaerobic work.

The most important experimental evidence presented by Embden (2) in support of his view was derived from experiments demonstrating the phenomenon which I shall call "the delayed formation of lactic acid." Two symmetrical isolated frog muscles were stimulated in the same circuit with a tetanus of say 5 seconds. One muscle was frozen in liquid air immediately on cessation of the stimulation. The other muscle was allowed to relax, and was not frozen till one to two minutes later. On analysing the muscles, it was possible to demonstrate that the lactic acid content in the muscle frozen first was always smaller than that in the muscle frozen a short time after relaxation. As the two muscles had been stimulated in exactly the same way, it seemed justifiable to assume that the content of lactic acid in the two was identical at the moment when the first muscle was frozen. Consequently, the increment in lactic acid found in the second muscle must have been formed after relaxation. This means that the formation of lactic acid does not coincide with the mechanical response, and, consequently, the formation of lactic acid cannot be directly associated with the mechanism of contraction.

This experiment was repeated on three different occasions by Meyerhof and his pupils (14, 19, 18) who, strangely enough, were unable to confirm the results. Meyerhof was convinced that no delayed formation of lactic acid takes place, and suggested that Embden's finding was due to a too strong stimulation of the muscles.

The view held by Meyerhof was mainly based on his demonstration of a constant ratio between the formation of lactic acid and the mechanical response of muscles working anaerobically (17). This ratio Meyerhof expresses in his isometrical coefficient of lactic acid: $K_{mL} = \frac{L \cdot S}{M}$. In this formula L means the length of the muscle, S the total tension developed during a series of twitches, and M the amount of lactic acid formed during the series of twitches. In English I think you would call the coefficient the Tension-length-lactic acid coefficient.

Moreover, Meyerhof found a fairly constant ratio between the heat formation in muscles and the formation of lactic acid, namely,

an output of 380 cal. per gram of lactic acid (13). The results of these two series of experiments therefore seemed to be consistent with the outcome of Hill's experiments, that the ratio between work and heat production is constant (6).

However, there remained a discrepancy between the heat formation per gram of lactic acid formed in the living muscle, and the heat liberated by the hydrolysis of glycogen to lactic acid and the neutralization of the acid formed by proteins. These processes should yield only 280 cal. per gram of lactic acid formed (15). It was therefore necessary to assume, either that the formation of lactic acid in the living muscle gave more heat than could be accounted for, or that its formation was accompanied by an unknown exothermic process, running closely parallel to it and giving the additional 100 cal. per gram of lactic acid formed. But even if this latter explanation of the discrepancy between directly measured and calculated heat should be the right one, the constant ratio between mechanical response and formation of lactic acid would be indicative of an intimate connection between the formation of lactic acid and the mechanism of contraction.

Meyerhof's well-known demonstration of the aerobic resynthesis of lactic acid into glycogen (12) gave support to the view he had adopted. Since lactic acid formed during anaerobic work is rebuilt into glycogen when the muscle is supplied with oxygen, it is possible to assume that during aerobic work, when no lactic acid accumulates, lactic acid is still formed to the same extent as would have been the case if the work had been carried out under anaerobic conditions. The demonstration of the aerobic resynthesis of lactic acid was in fact necessary for the proposition that, under all circumstances, even under aerobic conditions, the ratio between formation of lactic acid and mechanical response is constant.

The very important discovery of creatine phosphoric acid a few years previously by Fiske and Subbarow (4) and by Eggleton and Eggleton (1) strangely enough had not influenced the views held by Embden and Meyerhof. Though according to Embden phosphoric acid played an important part in the chemistry of muscular contraction, I think it justifiable to say that the discovery of creatine phosphoric acid was completely neglected by Embden. Meyerhof, however, at once made use of this new compound in his investigations.

It was shown by Meyerhof and Lohmann (16) that the breakdown

of creatine phosphoric acid (c-p-a) is an exothermic process giving 120 cal. per gram of phosphoric acid liberated. Meyerhof first thought that the breakdown of c-p-a was the unknown process, mentioned above, which ran parallel to the formation of lactic acid and gave the surplus of 100 cal. per gram of lactic acid formed. Further investigations, however, seemed to disprove this.

Nachmansohn (18) demonstrated that the anaerobic breakdown of c-p-a during a series of twitches does not run parallel with the work performed, but is greater in the first part of the series and then gradually decreases. This may be expressed as follows: The tension-length-c-p-a coefficient is smaller in the beginning of a series of twitches than later on; or, to put it in a simpler way, the amount of c-p-a broken down per unit of work performed decreases through a series of anaerobic twitches. Moreover, Nachmansohn (18) showed that creatine and phosphoric acid can be rebuilt into c-p-a under anaerobic conditions. This very important fact was demonstrated in experiments of exactly the same type as those of Embden demonstrating the delayed formation of lactic acid. In the muscle frozen immediately at the cessation of the stimulation a greater amount of c-p-a has been broken down than in the muscle frozen a short time after relaxation. The only way to interpret this finding is that part of the creatine phosphoric acid broken down during the tetanic contraction is rebuilt after relaxation.

Obviously, these results were not consistent with the experimental results used by Meyerhof to support his own conception against that of Embden. An exothermic reaction with an intensity decreasing through a series of twitches, occurring in conjunction with a constant formation of lactic acid, would give a greater total heat formation per unit of tension in the first part of a series of twitches than later on; yet, according to Hill the ratio TL:H is constant throughout a series of twitches. Moreover, an anaerobic endothermic resynthesis of creatine phosphoric acid, following a tetanic contraction, could not be explained by Meyerhof, for, according to him, no exothermic reaction capable of yielding the energy necessary was known to occur during the period immediately following a tetanic contraction. This view was maintained by Meyerhof, in spite of Hill's and Hartree's demonstration of the delayed anaerobic heat.

Since an exothermic breakdown of c-p-a did not fit into Meyerhof's general conception of the chemistry of muscular contraction, he suggested that c-p-a is not broken down in the working muscle but only transferred into a labile form which, in the chemical determinations, behaved like free creatine and free phosphoric acid. According to this suggestion, the change in c-p-a during muscular contraction is not an exothermic reaction, and consequently the c-p-a does not play any part in the liberation of energy. At present this theory seems hardly convincing, but we must admit that for Meyerhof at that time it supplied the only possible explanation.

I think I am justified in saying that the introduction of iodo-acetic acid poisoning (8) into the investigations on the chemistry of muscular contraction has been of some value in this respect, and that the results

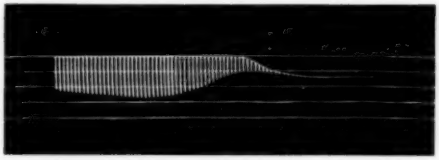

FIG. 1. Typical series of isometric twitches performed by a gastrocnemius muscle poisoned with iodo-acetic acid. Muscle weight: 0.7, g. Distance between two horizontal lines: 200 g. tension.

obtained by this method have made it absolutely necessary to reconsider the views generally held in 1929 upon the chemistry of muscular contraction.

Iodo-acetic acid inactivates the enzyme system involved in the formation of lactic acid. A muscle poisoned with this compound is capable of performing a series of 60–90 twitches anaerobically without any formation of lactic acid taking place. The muscle then goes into rigor (fig. 1). The twitches are completely normal in respect of latent period, duration of the twitch, heat formation, development of tension and action current (5). The total amount of work which an isolated poisoned muscle is able to perform is, however, smaller than that which a normal one can do. This may be expressed by saying that the energy reserve available under anaerobic conditions is decreased when the ability to form lactic acid is abolished. These facts

alone give a clear conception of the rôle played by the formation of lactic acid in the chemistry of muscle contraction. The formation of lactic acid is not a necessary chemical link, and consequently cannot have any direct connection with what I have called the mechanism of contraction. On the other hand, the formation of lactic acid or the ability to form lactic acid must be looked upon as an important energy reserve available under anaerobic conditions.

When we determine the chemical changes occurring in an iodoacetic poisoned muscle during stimulation, we find that the intensity of the breakdown of c-p-a is greater than in a normal muscle (8, 9). When a poisoned muscle has performed a series of 60–90 twitches, the muscle is exhausted and goes into rigor. At this stage the total amount of c-p-a has been broken down and, moreover, the adenylic phosphoric acid also has been broken down. In a normal muscle only about one-third of the c-p-a has been broken down after the performance of 60–90 twitches. In experiments on gastrocnemii, a series of about 80 twitches corresponds to a development of a total tension of approximately 30000 grams.

The first question to be answered is: from what chemical reaction does the energy liberated during the anerobic work of a poisoned muscle originate? Undoubtedly the energy must originate from the breakdown of c-p-a. In this connection it must be mentioned that in the poisoned muscles c-p-a is really broken down, and not merely transferred into a labile form as suggested by Meyerhof. The phosphoric acid set free by the breakdown is found in a new combination, namely, as hexose phosphoric acid and not as free phosphoric acid.

If the ratio between work performed (tension-length) and c-p-a broken down is determined in poisoned muscles, it is found to be fairly constant, regardless of the length of the series of twitches performed by the muscles. This, however, holds good only if the muscles are stimulated to a moderate extent, that is, to an extent at which no rigor develops. If they are stimulated to exhaustion and development of rigor, the ratio of tension-length to c-p-a broken down increases (11). This must mean that in the last part of a series of contractions another energy-yielding process starts. When the breakdown of adenylic phosphoric acid is traced, it can be demonstrated that the breakdown of this compound does not start till the muscle has performed about

two-thirds of the total amount of work it is able to perform, and rigor has begun to develop.

From these facts I think it is justifiable to conclude that the main source of energy and, in the first two-thirds of a complete series of twitches, the only source of energy is the breakdown of c-p-a. The breakdown of adenylic phosphoric acid, however, may also yield energy which can be utilized for the performance of work.

If the heat liberated in a poisoned muscle stimulated to a moderate extent is calculated from the amount of c-p-a broken down, and the tension-length: heat coefficient is calculated, the calculated coefficient will agree fairly well with the coefficient found in normal and poisoned muscles by direct myothermic determinations. Should there be anybody not convinced by these facts that the breakdown of c-p-a yields the energy liberated in the poisoned anaerobically working muscle, and yields energy which can be transferred into mechanical energy, *i.e.* utilized for the performance of external work, I may add the following experimental results.

The heat liberated during a tetanic contraction of a certain duration increases with the temperature at which the tetanic contraction is performed. Feng (3), in Hill's institute, has determined these variations by means of myothermic measurements, using normal muscles. If the heat formation is plotted against the temperature, we get a curve like the fully drawn curve in our figure 2. If similar experiments are performed with poisoned muscles (11), and the breakdown of c-p-a is plotted against the temperature, we get a curve like the broken curve in the figure.

These experimental results having been obtained in experiments on poisoned muscles, it was quite natural to conclude that the breakdown of c-p-a in the normal muscle is also an energy-yielding process, which partakes in the liberation of energy during anaerobic work. However, no agreement between this conclusion and the experimental results on which Meyerhof based his conception of the chemistry of muscular contraction seemed possible. You will remember that the main obstacles to the assumption that c-p-a is really broken down in the normal muscle were (a) the constant ratio between tension-length and lactic acid formation, together with the well-established constancy of the ratio between tension-length and heat, and (b) the lack of an

exothermic reaction, following a tetanic contraction, which might yield the energy necessary for an endothermic resynthesis of c-p-a.

These obstacles were, however, soon cleared away. New experiments showed that the ratio between tension-length and formation of lactic acid is not constant throughout a series of twitches, but larger in the first stages than later on. This means that the amount of lactic acid formed per unit of tension-length is smaller in the beginning of a series of twitches and gradually increases through such a series (10). You will remember that the breakdown of c-p-a varies in the opposite direction, being greater per unit of tension-length in the first part of a series of contractions. Obviously, the increase in the

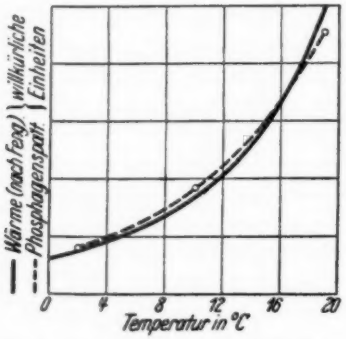

FIG. 2. Heat formation, and breakdown of creatine phosphoric acid per unit tension-length-time during tetani performed at different temperatures. Breakdown of creatine phosphoric acid and heat formation in arbitrary units.

amount of lactic acid formed per unit of tension-length balances the decrease in the breakdown of c-p-a, making the sum of these two processes, expressed in heat units, constant. The second obstacle, too, was soon removed. Embden's observation that lactic acid is formed in the period following immediately after a tetanic contraction was confirmed. Actually, the amount of lactic acid formed after relaxation is quite considerable. In my own experiments on this point (10) I have tried to determine quantitatively the delayed formation of lactic acid and the resynthesis of creatine phosphoric acid in the same muscles. My results have shown that the amount of energy calculated from the delayed formation of lactic acid corresponds fairly

well to the amount of energy which, according to calculation, is bound in the endothermic resynthesis of c-p-a. This explains why the delayed anaerobic heat is only a rather small fraction of the initial heat. The fact is, that the delayed formation of lactic acid is so pronounced that one would expect a liberation of a rather large amount of heat as delayed anaerobic heat, if the formation of lactic acid were not associated with an endothermic reaction. I may mention that at low temperatures (2°–0°C.) practically all the lactic acid formed in connection with a tetanus of a duration of 15–20 seconds is produced after the relaxation.

The results of the renewed experiments on the formation of lactic acid during anaerobic work are, in fact, only consistent with the results obtained by direct heat measurements, if we assume that the breakdown of c-p-a in the normal muscle is an exothermic process which must be taken into account in the energy balance sheet of the anaerobically working muscle. For this reason these experiments may be considered as affording strong evidence in favour of this assumption.

As already mentioned, the breakdown of adenylic phosphoric acid in poisoned muscles undoubtedly partakes in the liberation of energy during the last part of a series of twitches, and this energy is undoubtedly utilized for the performance of external work. For this reason it seems justifiable to conclude that a breakdown of adenylic phosphoric acid in the normal muscle may also yield energy which can be utilized for the performance of work. In normal muscles, however, a breakdown of adenylic phosphoric acid cannot be demonstrated. Only if the muscles are stimulated to complete exhaustion, or in some way injured, is a breakdown of adenylic phosphoric acid demonstrable. This does not necessarily mean that adenylic phosphoric acid is not broken down under normal conditions, since a breakdown may be followed immediately by a complete resynthesis. The energy balance sheet, of course, is not affected by a reaction of this kind.

According to my conception, three different sources of energy are available in the anaerobically working muscle, namely, the breakdown of the three compounds: adenylic phosphoric acid, creatine phosphoric acid, and glycogen. The absolute amounts of energy which can be set free by these reactions per gram of muscle are approximately 0.12, 0.30 and 1.0 cal., respectively.

If finally I should make an attempt to answer the question: what is

the mutual relationship between these three anaerobic energy-yielding reactions? We must to some extent leave facts and venture upon hypotheses.

A quite simple and primitive conception would be that the three reactions might be independent of each other. An anaerobically working muscle might derive the energy necessary under some circumstances from a breakdown of c-p-a, but under other circumstances from the formation of lactic acid from glycogen. In this case the chemical changes found in a muscle, after it has been stimulated under anaerobic conditions for some time, are a true and quantitative expression of the chemical reactions which have taken place. According to this conception, adenylic phosphoric acid never participates in the chemical reactions in the working muscle under normal conditions. Breakdown of c-p-a yields energy particularly in the first part of a series of twitches, *i.e.*, just at the start of anaerobic work, but gradually the production of energy is shifted over to lactic acid formation, the two reactions going on simultaneously, the breakdown of c-p-a with rapidly decreasing intensity, the formation of lactic acid with increasing intensity. This conception, according to which one reaction may act as a substitute for another, is quite simple, but cannot be upheld any longer, if not for other reasons than for the simple one, that it is now well known that a transference of phosphate from adenylic phosphoric acid to carbohydrate is a necessary link in the glycolytic breakdown of glycogen.

According to another conception, the three processes form a chain of reactions through which energy can be transferred to what I have called the mechanism of contraction. Energy liberated by the formation of lactic acid is transferred to c-p-a through a resynthesis of creatine and phosphoric acid to c-p-a. Again, the energy liberated by the breakdown of c-p-a is transferred to adenylic phosphoric acid by causing a resynthesis of this compound; and finally, energy liberated by the breakdown of adenylic phosphoric acid is transferred in some unknown manner to the mechanism of contraction. In this case the chemical changes found in a muscle after stimulation do not give a true picture of what has actually happened in the muscle during work, but only give a picture of the final result of a series of endo- and exothermic coupled reactions. In support of this view it may be em-

phasized that an anaerobic resynthesis of c-p-a has been directly demonstrated in the living muscle, a resynthesis which coincides with a formation of lactic acid, and for which the exothermic formation of lactic acid must yield the energy necessary. A synthesis of adenylic phosphoric acid coupled with a breakdown of c-p-a has been demonstrated in muscle extracts (7). That this reaction may proceed also in the opposite direction complicates matters, but at this junction I shall not go into details as to these enzymatic reactions studied in muscle extracts. If the breakdown of adenylic phosphoric acid cannot be demonstrated in the living muscle, the reason may be that the breakdown is always followed immediately by a complete resynthesis.

According to the latter view, the amount of c-p-a broken down in a normal anaerobically working muscle during the performance of a certain amount of work is exactly as large as the amount broken down in a poisoned muscle performing the same amount of work. In the normal muscle, however, only part of the c-p-a broken down remains broken down. The main part is rebuilt, thanks to the formation of lactic acid. The fraction of broken-down c-p-a which is rebuilt increases through a series of contractions, probably owing to the increasing concentration of free creatine and free phosphoric acid. For this reason the amount of c-p-a that remains broken down increases, first rapidly, and then more and more slowly.

Finally, I shall ask your permission to show you two quite simple diagrams, which are meant to give a picture of the two different conceptions of the mutual relationship between the three anaerobically available energy reservoirs in the muscle. Actually, I use these diagrams to make these conceptions clear to the students, but I hope you do not mind my using them here.

The energy reservoirs are pictured as water reservoirs with an outflow at the bottom. A is adenylic phosphoric acid, B creatine phosphoric acid, and C glycogen. Figure 3 represents the first conception. There are no connections between the different reservoirs, but water (energy) can be run from the reservoirs independently to the mechanism of contraction represented by the turbine. Normally, reservoir A is not used at all. Reservoir B is used to start the turbine, but very soon the outflow from reservoir C is opened, and, as this outflow is opened more and more, the outflow from B is gradually

closed. The fall of the water level in the different reservoirs is a direct measure of the amount of water which has passed through the outflow.

Figure 4 represents the second conception. The reservoirs are connected through tubes. From reservoir A water can run directly to the turbine, but from B it has to pass through A, and from C it has to pass through B as well as through A. By the lever mechanism the inflow to A from B is adjusted in such a way that the level in A is kept constant as long as there is water left in B. When the level in reservoir B (the c-p-a reservoir) falls to a certain level, the connection

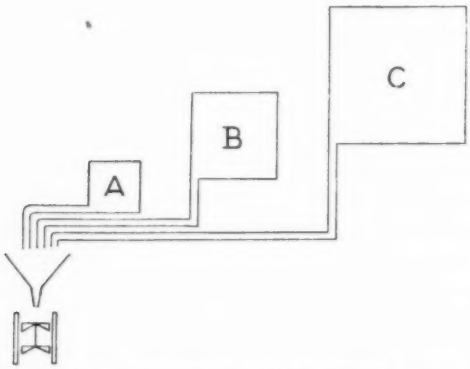

FIG. 3. Diagram showing conception I of the mutual relationship of the anaerobic energy-yielding processes.

with reservoir C (lactic acid formation) is opened, and opened more and more as the level in B falls, as indicated by the lever system. The level in the creatine phosphoric acid reservoir therefore will fall rapidly at first, then more and more slowly. If the outflow from the creatine phosphoric acid reservoir is very fast, as during a tetanus, the level in the reservoir falls so rapidly that the inflow from reservoir C (lactic acid formation) cannot keep pace with it, and only after the tetanic contraction has stopped is the creatine phosphoric acid reservoir filled up.

What I have said in this lecture is based on experiments carried out some years ago. There has not been anything actually new in it.

The conception of the chemistry of anaerobic muscular contraction which I have presented to you today is not shared by all physiologists working in this field. Particularly is it denied by some that the breakdown of c-p-a partakes in the production of energy in the anaerobically working muscle. I am grateful that I have had the opportunity personally of laying before you the conception of these problems to which principally the experiments in muscles poisoned with iodoacetate have led me, and I hope that the historical background which I

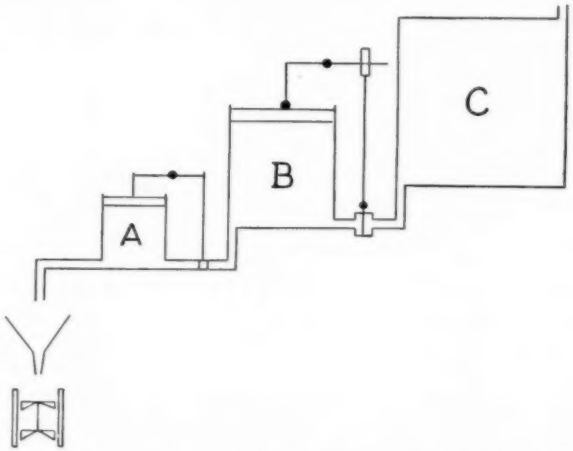

FIG. 4. Diagram showing conception II of the mutual relationship between the anaerobic energy-yielding processes.

have tried to give my view has been of some value for the understanding of how I arrived at this view.

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THE METABOLISM OF THE AEROBIC WORKING MUSCLES[1]

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It is an assumption frequently encountered in physiological literature that the metabolism of the aerobically working muscles is an exclusively carbohydrate metabolism. This assumption cannot have originated from experience gained from metabolism determinations during muscular exercise. It is a familiar fact that even heavy muscular exercise can be performed on a diet of fat alone, and with a respiratory quotient which indicates a combustion almost entirely of fat. Experiments by Krogh and Lindhard (6), and recently by their pupils (2, 3), have demonstrated this very convincingly. Of course, the fact that hard muscular work can be performed under conditions where the respiratory quotient is low does not disprove the belief that the working muscles oxidize only carbohydrate; for the possibility certainly exists that in the organism—presumably in the liver-carbohydrate is formed from fat, so that the muscles may very well be supplied with carbohydrate even when the organism as a whole is metabolizing fat alone. However, this purely hypothetical possibility can scarcely explain why it is so difficult to overthrow the conception of a purely carbohydrate metabolism in the aerobically working muscles.

If we endeavour to find out how such a conception arose, and whence it has obtained sustenance all this time, we shall quickly find that the conception of a purely carbohydrate metabolism in the striated muscles arises from, and is supported by, the experience gained in experiments on the chemistry of isolated muscles, mainly the isolated muscles of cold-blooded animals.

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The first to make convincing demonstrations of the connection between the mechanical function of muscle and a production of lactic acid, *i.e.*, a carbohydrate metabolism, were Fletcher and Hopkins (5). At this juncture, however, perhaps I may be permitted to say that presumably the first to express the supposition of a connection between a formation of lactic acid in the muscles and muscular work was the Swedish chemist Berzelius. Du Bois Reymond (4) tells us how in 1841 Berzelius stated that he had noticed that muscle from animals which had been hunted before being killed seemed to contain very considerable quantities of lactic acid, whereas muscle from partly paralyzed extremities seemed to contain only small quantities of that substance. Nevertheless, it was of course the famous investigations of Fletcher and Hopkins that brought lactic acid formation into the prominent position in muscle chemistry which it has since preserved. Their experiments showed how lactic acid, which had accumulated in muscle during work, would disappear after the work when the muscle was kept under aerobic conditions. However, the explanation of the disappearance of the lactic acid under aerobic conditions stands to the credit of Meyerhof (11), from whose fine experiments it appeared that under aerobic conditions lactic acid can be rebuilt into the glycogen from which it was originally formed. In forming an estimate of the value of these investigations, admirable as they are in themselves, in their more general biological applicability, it must be remembered that the direct demonstration of the resynthesis of glycogen from lactic acid can be made only under conditions where the formation and the removal of the lactic acid are separated in time. Finally, it must be borne in mind that, judging from these experiments—on cold-blooded animals only, it is true—the resynthesis of lactic acid to glycogen seems to be a fairly slow process. It is now easy to see why Meyerhof's demonstration of a carbohydrate cycle, glycogen⇄lactic acid, has become generalized, in the sense that such a cycle has been accepted as an explanation both of the so-called Pasteur reaction in yeast, and of the parallel circumstance that no lactic acid formation can be demonstrated in aerobically working muscles. If the theory should be maintained that a breakdown of glycogen to lactic acid is an absolutely essential phase in the chemistry of muscle contraction, it would become absolutely necessary to assume

that a formation of lactic acid takes place also in aerobically working muscles, indeed a lactic acid formation of the same degree as that taking place in a muscle which, under anaerobic conditions, works with the same intensity. In actual fact, the entire conception of the central rôle of lactic acid in the chemistry of muscle contraction only became possible, when Meyerhof's demonstration of the carbohydrate cycle permitted the assumption that the reason why no lactic acid formation can be demonstrated in an aerobically working muscle is, that the lactic acid actually formed is rebuilt into glycogen as quickly as it is formed. That the theory concerning the rôle of lactic acid formation in muscle contraction (for which Meyerhof fought so energetically, especially in his controversy with Embden) necessitated this interpretation of the Pasteur-Meyerhof reaction in the muscles, and thereby made the same interpretation of the Pasteur reaction in yeast probable, is undoubtedly the psychological reason why this interpretation was accepted without hesitation. For actually, the direct proofs of the correctness of this view are not strong.

By the Pasteur reaction is understood, as you know, the circumstance that under aerobic conditions fermentation recedes, in some cases entirely so that the yeast has a metabolism that is purely oxidative. As far as we can see, Pasteur himself conceived fermentation and oxidation as two mutually independent metabolic processes, in the sense that they are not coupled processes, but two reactions which, so to say, can take each other's place. Nowhere does Pasteur express the supposition that the cause of the recession of fermentation under aerobic conditions should be, that the terminal products of the fermentation process, or a product formed intermediately, are resynthesized into carbohydrate. That interpretation is Meyerhof's.

That the so-called oxidation coefficient, or the Meyerhof coefficient, is of fairly constant value, has been regarded as evidence of a kind that Meyerhof's interpretation of the mechanism of the Pasteur reaction is correct, that is, as an evidence of a coupling between oxidation and resynthesis into carbohydrate. The oxidation coefficient is expressed by the ratio between the recession of fermentation or glycolysis under aerobic conditions, and the oxidation intensity. The determination of this coefficient is of course of value only when it concerns yeast or tissues in which a residue of the fermentation or

glycolysis persists under aerobic conditions. It must be pointed out, however, that this coefficient displays nothing like pronounced constancy. And in those instances in which it has been assumed that there is a conformity between the oxidation coefficient in yeast and tumour tissue and the oxidation coefficient in muscle, it must be pointed out that no such conformity has been proved. The oxidation coefficient for muscle has been calculated on the basis of experiments on the directly demonstrable resynthesis of lactic acid to glycogen in muscle which, after having worked anaerobically, is supplied with oxygen. In the first place, the ratio found in such experiments between "disappeared lactic acid" and oxidation (oxygen consumed during restoration) is not at all constant; and in the second place, the question of how the basal metabolism of the muscle in the very long period of recovery is to be accounted for in the calculation is a most uncertain one. Finally, it must be pointed out—and that is perhaps the most important point—that the calculations are made on the supposition that the resynthesis of lactic acid is the only endothermic reaction in recovering muscles in which the oxidation energy is consumed, whereas we now know that other endothermic reactions take place (resynthesis of creatine phosphoric acid). All in all, it must be said that the alleged constancy of the oxidation coefficient in various tissues does not contain any visible evidence that the Pasteur-Meyerhof reaction depends upon a coupling between a resynthesis of carbohydrate on the one hand, and oxidation on the other.

It has been asserted that the correctness of Meyerhof's interpretation of the mechanism of the Pasteur-Meyerhof reaction is proved by Meyerhof's experiments with yeast (12), the object of which was to show more directly an oxidative resynthesis to carbohydrate of the final product of the fermentation process, alcohol. Thus these experiments would correspond exactly to the demonstration of the oxidative resynthesis of lactic acid to glycogen in muscles during aerobic recovery. Whereas in Meyerhof's muscle experiments it has been possible to show directly an increase of the glycogen content in the muscles, proceeding parallel with the disappearance of the lactic acid, no corresponding direct demonstration has been made of an increase of the carbohydrate content in yeast respiring in an alcoholic medium. On the other hand, Meyerhof under such conditions found a very low

respiratory quotient, the respiratory quotient when yeast oxidizes alcohol usually being as low as 0.3. Meyerhof took this low quotient to be evidence of a resynthesis of carbohydrate, a reaction having the character of a partial oxidation and therefore bound to give a low respiratory quotient. From the respiratory quotient Meyerhof calculated the ratio between oxidized and resynthesized alcohol, that is to say the "oxidation coefficient," and found that it was of the same value as the oxidation coefficients in yeast calculated in other ways and the corresponding coefficients in tumour tissue and muscle.

I have made experiments of the same kind (10), and have been in a position to confirm the regular occurrence of very low respiratory quotients when yeast respires in an alcoholic medium; at the same time, however, I consider that I have been able to show that Meyerhof's interpretation of these very low quotients is incorrect.

If a moderate quantity of alcohol is employed in the medium, and the experiments are continued for some hours, it will be found that in the first periods the respiratory quotient is very low. After a certain interval of time, dependent on the amount of alcohol added to the samples, the respiratory quotient rises rather suddenly and reaches unity. It can now be shown that as long as the respiratory quotient remains low, an organic acid accumulates in the samples, and, when the quotient rises, the organic acid begins to disappear while simultaneously the intensity of oxygen absorption begins to decrease. If the experiments are discontinued at a time when alcohol still remains in the samples, it is possible by direct alcohol determinations to establish the amount of alcohol that disappeared during the experimental period. From the carbon dioxide output and the directly determined acid formation (determined as increase in ether-soluble acids), it is possible to calculate the total amount of alcohol oxidized completely and of that oxidized to organic acid. If the sum of these two values is compared with the directly determined loss of alcohol, we find that the latter is somewhat greater than the sum of the other two values. Thus some alcohol seems to have disappeared which cannot be accounted for, and which consequently may have been converted into carbohydrate. This quantity, however, is much smaller in proportion to the alcohol oxidation than that calculated by Meyerhof. He calculated the ratio between alcohol resynthesized

into carbohydrate and alcohol oxidized at 3:1; according to my experiments the ratio cannot be more than 1:1, that is to say a ratio of dimensions quite different from the oxidation coefficient found previously. There can be no doubt that the very low respiratory quotients are mainly a result of the fact that alcohol is oxidized by yeast in two phases, the alcohol first being converted into organic acids (presumably acetic acid), which thereupon are oxidized completely. Consequently, nothing in these experiments tends to show that Meyerhof's interpretation of the mechanism of the Pasteur-Meyerhof reaction is correct, and therefore there is some justification in asking if there is any reason for continuing to accept that interpretation.

You will recollect my saying that Meyerhof's interpretation was, so as to speak, necessary if the conception of lactic acid formation as an essential part of the chemistry of muscle contraction were to be maintained. Here again it is not possible today to discern any reason for persisting in this interpretation.

At the present moment it is doubtless generally acknowledged that the formation of lactic acid is not essential to the chemistry of muscle contraction. I believe there is reason for saying that the introduction of iodo-acetic acid poisoning in muscle chemistry (8) has contributed very considerably to the rapid and complete acceptance of that view. Some of the results obtained in experiments on muscles poisoned with iodo-acetic acid I mentioned in my lecture yesterday. Another problem, not yet mentioned, has been elucidated through experiments of this kind (9). This problem, of essential importance to the subject of my lecture today, concerns the question whether an intermediate formation of lactic acid must be considered necessary for the utilization of oxidation-energy in working muscles, or, if you prefer, whether lactic acid formation is necessary for the transference, to the mechanism of contraction, of energy originating from oxidations.

When two symmetrical sartorii, which have been poisoned by soaking in Ringer solution to which iodo-acetic acid has been added, are stimulated, one in oxygen and the other in nitrogen, it is seen that the muscle supplied with oxygen is capable of performing much more work than the muscle stimulated in nitrogen. The latter will be completely exhausted and go into rigor at a time when the other muscle is still giving maximum tension. This is illustrated by records made in

experiments of the kind I have described (fig. 1). If the muscle which has worked aerobically is analysed at a time when it has performed a much greater work than the completely exhausted anaerobic muscle, it will be found still to contain considerable quantities of creatine phosphoric acid. This means that there can be no doubt that energy arising from oxidation processes can be utilized in the production of mechanical energy, that is to say, it can be utilized for the performance of exterior work, even under circumstances where there can be no question of intermediate lactic acid formation. That such a

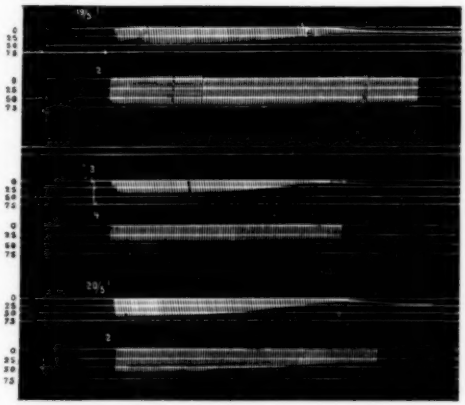

FIG. 1. Series of isometric twitches performed by IAA-poisoned symmetrical sartorii muscles, one stimulated in nitrogen (upper one) and one stimulated in oxygen (lower one).

formation is out of the question, may be concluded with certainty from the fact that the contralateral muscle (fig. 1), working under anaerobic conditions, passes through the series of twitches in a manner characteristic of a muscle completely poisoned with iodo-acetic acid.

We cannot say with certainty whether under these circumstances the oxidation energy is utilized through an oxidative resynthesis of creatine phosphoric acid, or whether it is transferred more directly to what we may call "the mechanism of contraction." As long as it has not been shown that the breakdown of creatine phosphoric acid is not a necessary link in the chemistry of muscular contraction, I shall

consider it most natural to assume that the oxidation energy is utilized through an oxidative resynthesis of creatine phosphoric acid; but perhaps the day is not far distant when it will be possible to show that the breakdown of creatine phosphoric acid is not an obligatory process in the chemistry of muscle contraction, just as it has been possible to show this as far as lactic acid formation is concerned.

To my mind the following conclusions are justified from the results obtained in experiments with muscle poisoned with iodo-acetic acid.

The formation of lactic acid is not an essential element in the chemistry of muscle contraction, nor is intermediate lactic acid formation a necessary element in the utilization of oxidation energy for the production of mechanical energy in the muscles.

Accordingly, the experience gained in experiments with isolated muscle no longer provides a reason for assuming that in aerobically working muscle there is a constant intermediate formation of lactic acid which, as quickly as it is formed, is resynthesized to glycogen. Another way of expressing this is, that no reasons can be found for accepting Meyerhof's interpretation of the Pasteur-Meyerhof reaction in aerobically working muscles. On the other hand, we may still speak of a Pasteur-Meyerhof reaction in aerobically working muscle, for by this we merely understand that the formation of lactic acid recedes in the muscles under aerobic conditions.

The question may now be asked: If we cannot accept the factors which originally supported the "classic" view of the Pasteur-Meyerhof reaction in the striated muscles, then are there any other arguments to be advanced in favour of that view? I do not think so. There are experiments on the blood lactic acid concentration in man during and after muscular exercise, and these experiments to my mind rather argue against the classic view of aerobic muscle metabolism. Experiments of this kind were made by O. Bang, partly in Krogh's institute and partly in mine (1).

By means of a micro-method, which makes it possible to measure the lactate concentration in blood samples of only 0.1 cc., Bang has followed the variations in the blood lactate concentration during and after exercise. Thanks to the method employed, the concentration could be determined at short intervals, whereby a reliable curve was obtained for the variations. As far as the present subject is con-

cerned, the greatest interest attaches to those experiments in which the working intensity was such that a "steady state" was quickly arrived at. The changes in the blood lactate concentration observed in experiments of this kind may be described as follows. Immediately after the commencement of the exercise the blood lactate rises quickly and reaches a maximum in the course of from seven to ten minutes. Then the concentration begins to fall, even when the work is continued. This means, then, that even after a steady state has been reached for oxygen absorption, this does not happen for the lactic acid concentration, at any rate, not in the blood. In experiments in which the working intensity is moderate, the blood lactate falls even to basal values during the work. Even if we cannot assume that the blood lactate at any moment reflects the concentration in the working muscles, the falling blood curves to me are incompatible with the conception of a high constant level in the working muscles throughout the working period. Especially does this apply to the experiments in which the blood lactate gets down to basal values during the work. Now, this high and constant level is necessary if the classic view of the metabolism of the aerobically working muscles is to be upheld. According to that view, the assumed constant lactic acid production was supposed to lead in relatively few minutes to the attainment of a constant increased lactate level in the active muscles, subsequently maintained throughout the "steady state." The constant level, it was thought, was attained by the rise in lactate concentration in itself inducing a rise in the intensity of oxidation, which in turn would involve a rise in the lactate resynthesis, until an equilibrium was established between production and resynthesis, at a level depending on the intensity of lactic acid formation, *i.e.* the intensity of work.

Personally, I am inclined to believe that Bang's interpretation of the experiments may be correct. According to Bang, we must assume that an intensive formation of lactic acid takes place in the active muscles in the first minutes after the commencement of work. Presumably the reason is that it takes a certain time for the general and local circulations to adapt themselves to the increased demand for oxygen. Until the circulation is adjusted, the muscles work partly anaerobically, that is to say they utilize the anaerobically available energy depôt represented by the ability to form lactic acid. When the

circulation is adjusted and the oxygen supply to the muscles becomes adequate, all formation of lactic acid ceases. Thus, at the commencement of work a "depôt" of lactic acid is formed in the active muscles. The size of this depôt is independent of the duration of the work, and solely dependent on its intensity. The greater the intensity of the work, the greater will be the quantity of lactic acid formed before the circulation has become adjusted.

Though the interpretation of the experiments cannot be said to be quite certain, I think one is justified in emphasizing the following: the fact that the blood lactate concentration may fall and may even reach basal values during the period of work is incompatible with the classic view, according to which the metabolism of aerobically working muscles includes an obligatory intermediate lactic acid formation which is balanced by an oxidative resynthesis of the lactic acid formed, when the lactic acid concentration in the muscles has reached a certain high level.

If now we return to the theory of a pure carbohydrate metabolism in the active muscles, it must be said that we have been unable to find any support for the theory in the experimental facts to which I have so far referred. This is a matter of considerable weight, as it eliminates the basis on which the theory to my mind has principally rested.

As I have laid particular stress on Meyerhof's interpretation of the Pasteur-Meyerhof reaction as a basis for the theory of a purely carbohydrate oxidation in aerobically working muscles, I ought to mention at the same time that this interpretation, as pointed out by Meyerhof himself, does not require as an inevitable consequence that the metabolism of the active muscles be a purely carbohydrate oxidation. There is the possibility that the energy necessary for the endothermic lactic acid synthesis is produced by the oxidation not of lactic acid or carbohydrate, but of fat or protein. This, however, does not alter the fact that Meyerhof's interpretation of the Pasteur-Meyerhof reaction has been regarded, and justly so I think, as an argument in favour of the assumption that the aerobically working muscles oxidize carbohydrate only.

Personally, I am inclined to the view that under such conditions, where a formation of lactic acid takes place in the active muscles, the effect is that the oxidation proceeds entirely, or mostly, at the expense

of carbohydrate. In muscle working partly anaerobically there will be simultaneously lactic acid formation and oxidation. With very intense muscular exercise, which can continue only for a short time, the muscles will work partly anaerobically, and under just such conditions we see respiratory quotients of about unity, even when in rest the quotient lies much lower. Exactly the same thing is seen in the untrained subject during moderate work. Here, too, the respiratory quotient rises and remains high during the work, and simultaneously the blood lactate curve rises steadily as a sign that the muscles are working under partly anaerobic conditions.

If it be true, as I am inclined to believe, that lactic acid formation in the active muscles always leads to a high respiratory quotient, that is to say to a combustion of carbohydrate, then it must be said that the low respiratory quotients seen in well-trained individuals on a fat diet, even during the performance of heavy work, also indicate that lactic acid formation is not an obligatory link in the metabolism of the active muscles.

In conclusion, I would refer to a matter which, to me, has been of no small importance in deciding whether there is reason for assuming that the metabolism of the aerobically working muscles is of specific character. For if it is, the most natural conclusion to draw is that it is a purely carbohydrate metabolism.

What I have in mind in this connection is the fact that the intensity of alcohol oxidation is not affected by muscular exercise.

I admit that the question whether muscular exercise affects the intensity of alcohol oxidation has been answered differently by various authors. Nevertheless, in recent years more and more have accepted the view that the question must be answered in the negative. Among the experimental results which seem to show that the intensity of alcohol oxidation is not increased during muscular exercise, those of Nyman and Palmlöw (13) are particularly convincing. As shown in experiments by Widmark (14) and by others, however, the intensity of alcohol oxidation increases if metabolism is stimulated by dinitrophenol. This is also the case with thyroxin, according to Le Breton's preliminary experiments (7). As alcohol oxidation represents up to 80 per cent. of the basal metabolism, it would seem necessary to assume that part of the alcohol oxidation takes place in the muscles.

Therefore, the circumstance that an increased metabolism conditioned by muscular exercise does not increase the alcohol oxidation, whilst an increased metabolism produced by stimulating drugs does so, seems to justify the conclusion that there is a specific difference between the metabolism of the resting muscles and that of the active muscles. In her monograph on alcohol oxidation Le Breton actually arrives at a conclusion to that effect.

The circumstance that muscular exercise does not increase the intensity of alcohol oxidation might, however, have the explanation that neither in rest nor in work is alcohol metabolized directly in the muscles. If we imagine that alcohol oxidation is a specific function of the liver, it is easy to understand that an increased metabolism, due to an increased energy output in the muscles alone, does not increase the alcohol oxidation, whereas an increase of the metabolism intensity in all tissues, the liver included, does involve an increase of the alcohol oxidation.

One difficulty in assuming that oxidation of alcohol takes place only in the liver, is that the absolute quantity of alcohol oxidized during rest, as already stated, represents up to 80 per cent. of the basal oxygen absorption, whereas in the liver the oxygen absorption amounts to only 30 or 40 per cent. of the total basal oxygen absorption. Therefore, the assumption that alcohol oxidation is a specific liver function is conditional upon the alcohol oxidation in the liver being a partial oxidation. *A priori* that idea cannot be dismissed, so much the more because partial oxidations are undoubtedly a characteristic feature in the metabolism of the liver. Even if of course we must also assume that the partially oxidized product formed in the liver is oxidized in all tissues, the muscles included, the primary oxidation in the liver will nevertheless be the limiting factor for the disappearance of alcohol in the whole system.

That the supposition I have sketched is undoubtedly right appears from certain experiments, as yet unpublished, which I have made on alcohol oxidation in artificially perfused isolated livers and hind-limb preparations. These experiments show that, in the liver, alcohol undergoes a rapid but primarily only a partial oxidation, whereas alcohol is not affected oxidatively at all in a hind-limb preparation. I shall take the liberty of showing you one or two curves from these experiments.

From these curves (fig. 2) it will be seen that the concentration of alcohol in the blood, when perfused through a liver, falls quickly and at quite a constant rate. In the course of 90 minutes the entire quantity of alcohol (300 mg.) will have disappeared. The quantity of oxygen necessary to oxidize 300 mgs. of alcohol completely is 440 cc. The oxygen absorption in the first 90 minutes was only 225 cc. After adding the alcohol, the respiratory quotient falls from 0.69 to 0.37. This extraordinarily low quotient can only be explained as being due to incomplete oxidations. The lack of conformity between the quan-

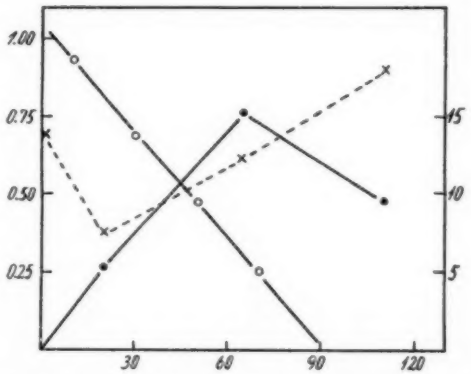

FIG. 2. Exp. on alcohol oxidation in isolated cat liver. Abscissæ: time in minutes. Left ordinate: blood alcohol in per thousand. RQ. Right ordinate: decrease in alkali reserve in cc. of carbon dioxide.

O———O Blood alcohol.
×-------× RQ.
·———· Acid formation (decrease in alkali reserve).

tity of alcohol lost and the quantity of oxygen absorbed may be explained in the same manner. In addition, the alkali reserve of the blood falls as a sign that acid products are being formed. When the alcohol has almost disappeared from the blood the alkali reserve begins to increase again. At the same time the respiratory quotient also rises; in the last determination in this experiment it reached a value of 0.9. The increase of both the alkali reserve and the respiratory quotient must be assumed to be the result of a complete oxidation of the partially oxidized acid products formed in the first period after

adding the alcohol. Incidentally, the similarity between an experiment of this kind and the yeast experiments previously mentioned is very striking.

The acid product formed in the liver experiments was not isolated and identified, but there can hardly be any doubt but that it was acetic acid. As acetic acid oxidizes with great rapidity in the living organism, it is assumable that the acetic acid formed in the liver of an

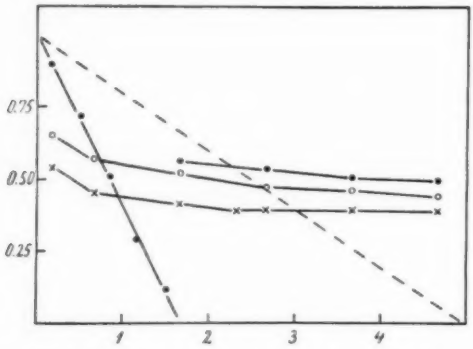

FIG. 3. Exps. on alcohol oxidation in isolated hind-limb preparations. Abscissae: time in hours. Ordinates: blood alcohol in per thousand.

The three curves from three different experiments on hind-limb preparations may be compared with a curve from an experiment on a liver (steep curve in the left part of the figure). As, however, the total amount of blood and tissue acting as solvent for the alcohol is three times as large in the hind-limb preparations as in the liver preparations, the curves from the experiments on hind-limb preparations actually must be compared with the broken curve.

intact animal after the addition of alcohol is carried with the blood to all the different tissues and oxidized there.

In an isolated hind-limb preparation alcohol is not affected oxidatively at all. After diffusion balance is established we see only a very slow fall in the blood alcohol concentration, a fall which does not exceed that seen in control experiments, in which the blood is circulated through the apparatus without passing living tissue (fig. 3).

The absolute quantity of alcohol oxidized in an isolated liver per gram of liver tissue per minute averages no more than 60 per cent. of the quantity oxidized in the entire animal per gram of liver tissue

per minute. Most probably the explanation for this is that the oxygen consumption of the liver, that is to say the metabolism intensity, falls distinctly in the course of the first five or ten minutes after isolation, so that we must also assume that the alcohol oxidation in a liver *in situ* is greater than that in an isolated liver. The dominating rôle played by the liver in alcohol oxidation also appears from the fact that, in eviscerated animals, the alcohol oxidation is extremely low and does not exceed 10 per cent. of that of intact animals.

Thus the assumption that the oxidative metabolism of the active muscles is of a specific nature cannot find support in the circumstance that the intensity of alcohol oxidation is not increased during muscular exercise. The explanation is that alcohol is primarily affected oxidatively in the liver, and it is the primary oxidation in the liver that becomes the limiting factor in the alcohol oxidation of the total organism.

In the course of this lecture I have referred to experimental results from very different fields within physiology: from research on the oxidative metabolism of yeast, from experiments with muscle poisoned by iodo-acetic acid, the concentration of blood lactate in man during muscular exercise and the metabolism of alcohol in the organism. To me, all these results, however different in nature they may seem, have been of significance in my consideration of the problem of the aerobic metabolism of muscle.

There is no doubt that this problem is capable of elucidation in many other and better ways. I have mainly quoted results from fields in which I myself have worked experimentally and which therefore have played a very special part for me in my deliberations. In my opinion, all the various relations I have submitted to you are conformable, in that they contain no basis for the assumption that the aerobic metabolism of the active muscles is a purely carbohydrate metabolism; indeed, some of them are directly contradictory. It is possible that some of you share the view I have arrived at from this, that the aerobic metabolism of the active muscles is not an exclusively carbohydrate metabolism, whereas others do not. I hardly imagine that I have convinced those who do not share my view, but it has been a source of great pleasure to me to have the opportunity of going so deeply into the considerations on which my view of the problem is founded.

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THE EFFECT OF HYPERTHYROIDISM UPON THE METABOLISM OF VITAMIN C

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Recent evidence that infectious diseases deplete the vitamin C supplies of the organism has led to considerable interest in the rôle which this vitamin plays. During febrile states and during convalescence the urinary excretion of ascorbic acid is greatly reduced. It has therefore been suggested that vitamin C plays a rôle in the immune reactions of the organism and that a low intake of vitamin C may render the individual more susceptible to infection (1).

The chemical nature of ascorbic acid suggests that the increased "usage" observed during infections may be related to the increased rate of metabolism rather than specifically to the infectious process. Since ascorbic acid is readily oxidized in neutral solution, its fate in the organism might possibly be influenced by the oxidative level or the metabolic rate.

Since an increased metabolic rate occurs in the absence of infection in individuals with hyperthyroidism, it was thought that a study of the effect of hyperthyroidism upon the metabolism of vitamin C would be of interest.

Five patients were studied before and after subtotal thyroidectomy. The vitamin C content of the diet was kept constant and the vitamin C excreted in the urine was titrated. A record was kept of the temperature, basal metabolic rate, and fluid balance before and after operation.

EXPERIMENTAL METHODS

All of the patients were studied on the Metabolism Ward of the Johns Hopkins Hospital. A special diet was prescribed and 24-hour urine specimens were collected. The patients remained on the ward for at least 14 days before and for 10 days after operation; and were readmitted for study six to eight months later.

A daily intake of 100 mgs. of Vitamin C was chosen. This quantity is twice the "minimal optimal" (2) and eight times the minimal antiscorbutic dose (3). Approximately one third of the total quantity was given with each meal as orange, tomato or grapefruit juice. An aliquot preserved with 10 per cent of 2-normal hydrochloric acid was kept in a brown bottle in a refrigerator and assayed daily. The rest of the diet contained very little vitamin C, inasmuch as fresh fruits, fresh vegetables, and raw milk were not allowed. When Lugol's solution was administered it was mixed with 30 ml. of grape juice. No saline medication was prescribed.

The urine was preserved with 10 per cent of 2-normal hydrochloric acid immediately after it was voided and kept in a brown bottle in a refrigerator. These precautions were observed because ascorbic acid is an unstable substance. Oxidation is inhibited in strongly acid solutions or in the presence of reducing agents. In slightly acid or neutral solutions the rate of oxidation depends upon the presence of light and of minute amounts of copper as well as upon the temperature and oxygen tension of the solution. In alkaline solutions, such as exist in the bladder at times, oxidation is rapid and irreversible (4) (5). For this reason the hydrogen-ion concentration of the freshly voided urine was tested. The patients on the above regime excreted an acid urine, the pH varying between 5 and 6.

The estimation of vitamin C was performed according to the method of Tillmans (6) as improved by King (7) with only slight modification. Hydrochloric acid was used to preserve the solutions and potassium acid phthalate was employed to bring the reaction back to pH 3 before titration.

The working standard was a solution of iodine in potassium iodide. Vitamin C assays were performed by titration with a 0.001-normal solution of the ether extracted sodium salt of 2,6,dichlorophenol indophenol. Both the dye solution and a standard solution of ascorbic acid, containing approximately 50 mgs. per liter and preserved with 10 per cent of 1-normal hydrochloric acid, were made up fresh weekly and stored in a refrigerator. The ascorbic acid solution was titrated each day with both the iodine standard and the dye solution; a figure for the strength of the dye solution being obtained in this manner. The urine and fruit juice, preserved with 10 per cent of 2-normal hydrochloric acid, were further diluted with 0.2-normal hydrochloric acid until the concentration of ascorbic acid was no greater than that of the standard solution. For the fruit juices this amounted to a tenfold dilution, which reduced the interference from coloring matter to a minimum. The hydrogen-ion concentration of all samples titrated was brought to approximately pH 3 by the addition of potassium acid phthalate from a burette. A few drops of thymol blue were added to an aliquot, and potassium acid phthalate added until the solution became orange in color. An equal amount of potassium acid phthalate was added to the other aliquots of the same sample. The dye solution was then added from a burette, 0.1 ml. at a time, until a definite pink color appeared which lasted 10 seconds and faded before a minute. Calculations were based upon the fact that ascorbic acid has two equivalents of reducing action per mol.

Although checked by animal assay (8) and by electrometric titration (9), this method has been questioned for its accuracy and validity. It seems that the method is accurate when the urine contains an appreciable amount of vitamin C and is reliable if no abnormal reducing agent is present. However, it must be emphasized that although one interprets the titrations in terms of mgs. of vitamin C, what is actually measured is the reducing capacity of the solution under the given conditions.

CASE HISTORIES

R. L. (Chart I), a 25 year old single white medical student, was known to be in good health. Physical examination (T:98.6°F, P:78, R:18, BP:120/70, Weight: 160 lbs.) was essentially normal. The basal metabolic rate was plus 14 per cent. His diet before starting the study was very high in vitamin C. During the brief interval in the middle of the study the intake of vitamin C was quite low.

L. E. (Chart II) U 104247, a 49 year old, single, white housekeeper, was admitted to the Johns Hopkins Hospital April 18, 1937 complaining of nervousness, weakness and loss of weight. One aunt had had a goiter removed. The patient had been well all of her life until five years ago when she had family difficulties and became very nervous. Three years ago she began to lose weight, menstruation ceased and her eyes became prominent. Since then she has noticed a swelling in the neck, dyspnoea on exertion, palpitation, extreme irritability and a tremendous intake of food and fluid. Physical examination (T:99.6°F, P:104, R:22, BP:160/60, Weight: 121 lbs.) at the time of admission revealed an agitated nervous woman with tachycardia, tremor of the hands, warm moist flushed skin, exophthalmos and abundant lachrymation. There was evidence of recent weight loss. A diffuse soft goiter was present. The heart was not enlarged, but a soft systolic murmuer and a gallop rhythm were audible. The spleen was palpable. There was a trace of acetone in the urine. The basal metabolic rate was plus 100 per cent. She was given bed rest, phenobarbital, chloral hydrate and continuous tub baths. Later Lugol's solution was given. Bilateral partial thyroid lobectomy was performed on May 7, 1937. For a few days after the operation the patient suffered from delusions, but then made a rapid recovery. She was discharged on May 22, 1937. Since discharge she has noticed a remission of all her symptoms except those concerned with her eyes. On readmission November 13, 1937, for study, she presented the appearance of a normal individual, somewhat overweight and with marked exophthalmos. (T:99.0°F, P:80, R:20, BP:120/80, Weight: 156 lbs.) At this time her basal metabolic rate was minus 2 per cent and her blood cholesterol 230 mgs. per cent.

C. G. (Chart III) U 102894, a 48 year old, white, married salesman, was admitted to the Johns Hopkins Hospital March 20, 1937, complaining of tremor of the hands and generalized weakness. His general health had been excellent until four months before admission when he became nervous and irritable. Three months before admission his work became hampered by a tremor of the hands and a feeling of weakness. Two weeks before admission, for the first time, he was

aware of breathlessness and palpitation after moderate exertion. In a period of four months he had lost 40 pounds in weight. Physical examination at the time of admission (T:99.4°F, P:106, R:22, BP:130/68, Weight: 165 lbs.) showed a restless nervous individual with staring eyes. He had slight fever and tachycardia. His pharynx was slightly inflamed. The thyroid was soft and large. The pulse was bounding and the heart was slightly enlarged with a systolic murmer at the apex. The basal metabolic rate was plus 58 per cent. He was given bed rest, and phenobarbital followed shortly by Lugol's solution. Bilateral partial thyroid lobectomy was performed on April 6, 1937. The patient made a rapid recovery and was discharged April 17, 1937. The next day he started work and has felt fine, troubled only by the prominence of his eyes and excessive lachrymation. He was readmitted November 20, 1937 for study. At that time physical examination (T:99.4°F, P:88, R:20, BP:140/90, Weight: 194 lbs.) was essentially normal save for the presence of marked exophthalmos and a persistence of the systolic murmer at the apex of the heart. His basal metabolic rate was plus 15 per cent and the blood cholesterol 234 mgs. per cent.

S. N. (Chart IV) U 104617, a 29 year old single, white, postman, was admitted to the Johns Hopkins Hospital April 4, 1937 complaining of shortness of breath, nervousness, a swelling in the neck and loss of weight. An uncle, his father and a brother had been treated for exopthalmic goiter. The general health of the patient had been good up until ten months before admission when he first noticed nervousness and began to lose weight. His other symptoms rapidly followed, increasing in severity until the time of admission. Physical examination (T: 99.5°F, P:88, R:20, BP:145/70, Weight:139 lbs.) showed a restless individual with prominent eyes and tremor of the hands. His skin was flushed and covered with perspiration. The thyroid was soft and enlarged. There was a systolic murmer audible over the entire precordium. The basal metabolic rate was plus 52 per cent. He was given bed rest and phenobarbital followed by Lugol's solution. A bilateral partial thyroid lobectomy was performed on April 30, 1937. Convalescence was complicated by a slight upper respiratory infection. He was discharged on May 11, 1937. Since then he has steadily gained weight, and a month after discharge he noticed a slight swelling of his face and extremities. Thyroid extract, ¼ grain three times a day was prescribed, and he took this up until one week before his readmission, November 25, 1937. At this time he presented a typical picture of mild hypothyroidism (T:99.0°F, P:60, R:18, BP: 115/80, Weight:168 lbs.) with a basal metabolic rate of minus 27 per cent and a blood cholesterol of 361 mgs. per cent.

B. M. (Chart V) U 77247, a 61 year old, white, married housekeeper, was admitted to the Johns Hopkins Hospital February 8, 1937, complaining of palpitation and swelling of the legs. Her sister and half brother as well as many of her neighbors in Wisconsin had goiters. She first noticed a prominence in the neck at the age of fourteen. For the past several years she had been getting increasingly nervous and uncomfortable, especially in hot weather. Two years ago she noticed swelling of her feet which has become more pronounced lately. During the course

of her illness she lost 35 pounds. Physical examination at the time of admission (T:99.4°F, P:102, R:20, BP:186/86, Weight:145 lbs.) revealed an elderly woman

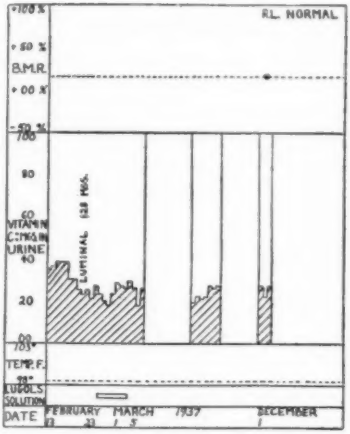

CHART I

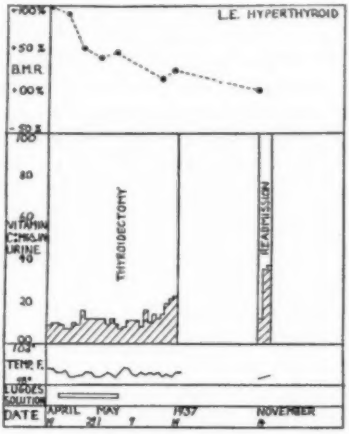

CHART II

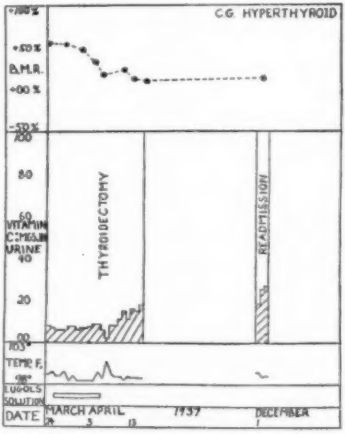

CHART III

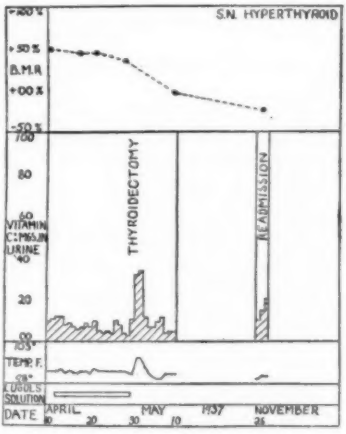

CHART IV

with moist flushed skin, engorged neck veins and a large firm mass in the region of the thyroid. In the latter a superficial nodule was palpated, which seemed to

extend below the sternum. The heart was enlarged to the left and a systolic murmer was heard at both apex and base. The pulse was rapid, the blood vessels thick and sclerotic, and the blood pressure elevated. There was extensive pitting edema of the lower extremities. The basal metabolic rate was plus 57 per cent. She was given bed rest and phenobarbital. Following this she was digitalized and then given Lugol's solution. A bilateral partial thyroid lobectomy was performed on March 12, 1937, 250 grams of thyroid tissue being removed. She made an uneventful recovery and was discharged on March 26, 1937. Two months later the patient had recovered her strength, started to gain weight and was free from cardiac symptoms. She was readmitted for study on November 28, 1937. At this time (T:99.0°F, P:76, R:20, BP:170/95, Weight:167 lbs.) there was no

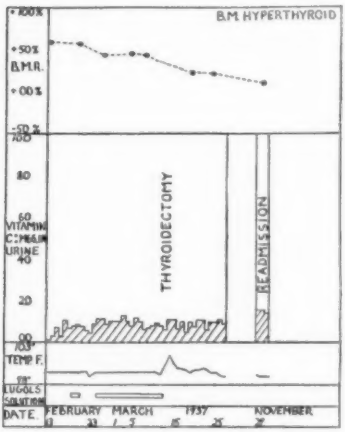

CHART V

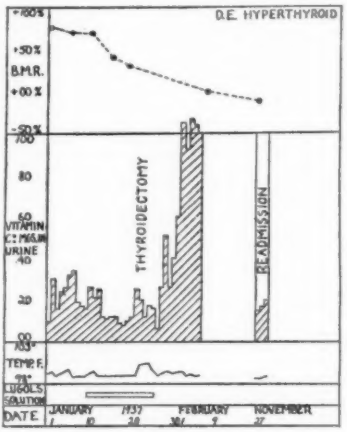

CHART VI

venous engorgement or peripheral edema. The heart was smaller than it was in February, although the murmurs persisted. Both the systolic and diastolic blood pressures were elevated. Her basal metabolic rate was plus 10 per cent and the blood cholesterol 272 mgs. per cent.

D. E. (Chart VI) U 127164, a 34 year old, white, married factory worker, was admitted to the Johns Hopkins Hospital December 29, 1936, complaining of nervousness, difficulty with her eyes and a swelling in the neck. The patient had enjoyed good health until an attack of scarlet fever at the age of 26. After this she lost weight and noticed nervousness and profuse perspiration. At that time members of her family noticed the prominence of her eyes and a swelling in her neck. Five years ago at the age of 29 she entered a hospital where she was given iodine and where a partial thyroidectomy was performed. After this she felt

better, gained weight and returned to work. During the past four years her old symptoms gradually returned. In addition she noticed breathlessness, palpitation and precordial pain, as well as finding it necessary to use several pillows at night. Physical examination at the time of admission (T:99.2°F, P:120, R:24, BP:160/90, Weight:128 lbs.) revealed a thin nervous woman with flushed face, prominent eyes, an enlarged thyroid, and tremor of the hands. Her heart was not enlarged but there was a systolic murmer at the apex and the rate was rapid. The urine showed a trace of albumin. The basal metabolic rate was plus 70 per cent. She was given bed rest and phenobarbital, later Lugol's solution. A bilateral partial thyroid lobectomy was performed on January 22, 1937. Convalescence was uneventful. After being discharged on February 7, 1937, she gradually regained strength and weight. She was readmitted for study on November 26, 1937, at which time physical examination (T:99.0°F, P:68, R:18, BP:145/85, Weight:165 lbs.) was essentially normal except for the presence of marked exophthalmos. Her basal metabolic rate was minus 13 per cent and the blood cholesterol was 219 mgs. per cent.

RESULTS

The vitamin C excretion of a normal individual, R. L. (Chart I), remained fairly constant on the controlled diet. There was a slight temporary drop immediately after the interval when the vitamin C intake was low. The effect of medication used in the treatment of hyperthyroid disease was studied in this individual. A single dose of 0.128 gms. of phenobarbital had no observable effect upon the excretion of vitamin C. Likewise a week of medication with Lugol's solution, 3 ml. three times a day, produced no change in the urinary excretion. The method of administering Lugol's solution is described under Experimental Methods.

L. E. (Chart II), whose basal metabolic rate was plus 100 percent at the time of admission, was found to have a daily excretion of about 10 mgs. Two weeks after operation when the basal metabolic rate was still elevated the daily excretion of vitamin C had doubled. Seven months later when the basal metabolic rate was normal the vitamin C excretion was four times as high as it had been before operation.

The record of C. G. (Chart III), is quite similar to the record of the first patient, L. E. The basal metabolic rate at the time of admission was plus 58 percent and the daily excretion of vitamin C less than 8 mgs. After subtotal thyroidectomy the vitamin C content of the

urine doubled. Eight months after operation when the patient's basal metabolic rate was plus 15 percent, his vitamin C excretion was more than three times as great as that observed during the preoperative period.

S. N. (Chart IV), was also found to have a very low level of vitamin C excretion before operation. Postoperatively his course was complicated by an upper respiratory infection and during convalescence he developed myxoedema for which he was given 0.0045 gms. of thyroid daily. Although his basal metabolic rate decreased from plus 48 percent to minus 27 percent the increase in excretion of vitamin C after operation was less marked than in the preceding two cases.

B. M. (Chart V), whose illness was complicated by chronic myocardial insufficiency, showed a remarkably low level of vitamin C excretion. Before admission her diet had been normal and she was in the hospital for four days before the study began. Nevertheless, during the first four days of the study her daily vitamin C excretion averaged less than 4 mgs. Immediately after subtotal thyroidectomy the vitamin C excretion did not change appreciably. Eight months after operation, when her basal metabolic rate had fallen from plus 57 percent to plus 10 percent, her average daily excretion of vitamin C was four times as great as during the first four days of the study. However this substantial rise in the excretion of vitamin C was not sufficient to bring it into the range of normal.

The vitamin C excretion of D. E. (Chart VI) followed an erratic course. The amount of vitamin C found in her urine fluctuated considerably, at times getting as low as 8 mgs. a day. After subtotoal thyroidectomy her basal metabolic rate dropped from plus 74 percent to minus 2 percent and the titer of reducing substances in her urine reached an extraordinarily high level which was not found during the brief check up period. However as with L. E., C. G., and S. N. the amount of vitamin C excreted during the check up period rose each day.

DISCUSSION

On the basis of urinary excretion of vitamin C individuals may be grouped into three classes; (A) normal persons who are found to excrete 20 to 50 mgs. daily (3) (10); (B) individuals, who because of a

period of diminished intake or because of a febrile reaction, excrete only 10 to 20 mgs. daily; (C) those individuals who have been deprived of the vitamin for several weeks, as well as individuals with scurvy who excrete less than 10 mgs. a day (11).

From these experiments it seems certain that the urinary excretion of vitamin C is greatly reduced in hyperthyroidism even when the intake is high. In four of the five patients studied the amount of vitamin C excreted during the period of hyperthyroidism was in the range observed in scurvy. Following subtotal thyroidectomy an increase in vitamin C excretion was observed in all cases, the increase being more than 300 percent in some cases, L. E., C. G., and B. M. In one patient only, B. M., did the rise in vitamin C excretion after hyroidectomy fail to reach the normal range.

Experiments with laboratory animals have demonstrated the effect of thyroxin upon the storage of vitamin C. The vitamin C content of the organs of thyroidectomized animals is greater than the vitamin C content of normal animals, which in turn is greater than that of animals receiving small amounts of thyroxin (12) (13) (14). It has been claimed that feeding large doses of vitamin C to animals that are getting thyroxin improves their appearance, lessens the weight loss (15) (16), and lowers the metabolic rate (17) (18).

SUMMARY

The vitamin C excretion of five hyperthyroid patients on a constant diet was studied before and after subtotal thyroidectomy. In all the patients the amount of vitamin C excreted before operation was far less than normal. Following thyroidectomy the vitamin C excretion increased in all the patients reaching a normal value in four of the five studied.

The author wishes to thank Dr. G. A. Harrop for planning and aiding these experiments; also Dr. G. W. Thorn and the personnel of the Metabolism Ward for their interest and assistance.

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URINARY CHANGES DUE TO SULFANILAMIDE ADMINISTRATION[1]

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Clinical acidosis due to the administration of sulfanilamide was observed by Southworth (1) who noted a drop in the CO_2-combining capacity of the plasma in fifteen successive cases treated with this drug. Marshall, Cutting and Emerson (2) have shown that in dogs this is a true acidosis with a lowering of the pH of the blood, but at the same time note that the pH of the urine rises.

The present study was undertaken to investigate the effect of sulfanilamide upon urinary constituents.

Three normal male subjects were provided with a diet of constant mineral composition and a constant fluid intake. A liquid diet was used in order to simulate the hospital diet provided for acutely ill patients. This consisted of 1000 cc. of grade A milk, 200 cc. of 40% cream, 1000 cc. of fresh orange juice and 500 cc. of water daily, together with 4 gms. of NaCl in capsules, equivalent to 68.4 m.eq. of sodium and of chloride. Two subjects were maintained on this constant metabolic regime for several days prior to the administration of sulfanilamide and, as an additional control, for a few days after it. The third subject was on the régime for a preliminary control period of only one day. Twenty-four hour urine specimens were completed at 7:00 A.M., preserved with toluol, and kept in a refrigerator. Sulfanilamide was administered at 8:00 A.M., 12 noon, 4:00 P.M. and 8:00 P.M., in an average dose of 4.8 gms. daily or about 0.1 gm. per kg. per day.

Upon completion of each 24-hour urine specimen the ammonia was determined without delay by the aeration and titration method of Folin as modified by Van Slyke and Cullen (3). Potassium determinations were made according to the method of Shohl and Bennett (4) on specimens ashed by the method of Strauss (5).

[1] This investigation was supported by a grant from the Winthrop Chemical Company, New York City.

Sodium was weighed as the uranyl zinc sodium acetate (6). Inorganic phosphate was analyzed according to the method of Fiske and Subbarow (7). Total nitrogen was determined by the macro Kjeldahl method. The open Carius method, as applied by Van Slyke (8), was used for the determination of chlorides. Urinary pH was determined colorimetrically using fresh buffer standards that were checked by the glass electrode.

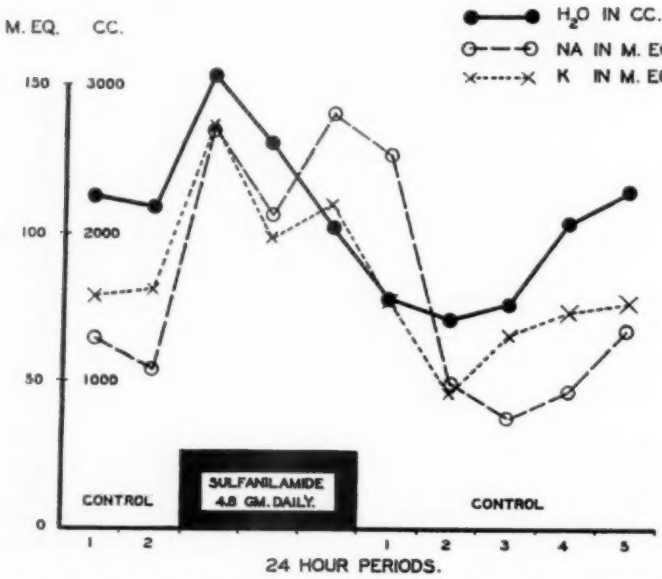

FIG. 1. URINARY OUTPUT OF WATER, Na, AND K IN PATIENT L. H.

Blood specimens were drawn daily from each subject. Sulfanilamide was determined by the method of Marshall (9) and the CO_2-combining power by the method of Van Slyke and Cullen (10).

The administration of sulfanilamide resulted in a marked increase both in the 24-hour urine volume and in the renal excretion of sodium and potassium (see Figure 1). In case L. H. the concentration of these cations per cc. of urine actually increased, while in cases C. D. and J. M. there was no significant change in their concentration. The quantity of free ammonia in the urine was not altered significantly (see Table I). A slight increase in the pH of the 24-hour specimens

TABLE I

Daily changes in urine and blood during sulfanilamide administration

SUBJECT	URINE*									BLOOD PLASMA	WHOLE BLOOD
	24-hour periods	Volume	Na	K	Cl	P (inorganic)	Ammonia N	Total N	PABS	pH	CO_2 combining capacity	PABS
		cc.	*m.eq.*	*m.eq.*	*m.eq.*	*gm.*	*gm.*	*gm.*	*gm.*		*vol. per cent*	*mg. per cent*
C. D.	Control	1,830	77.3	58.2	97.4	0.74	0.2	8.0		7.0		
	Control	2,035	84.8	58.6	102.6	1.00	0.3	9.9	0.0	6.7	63.2	0.0
	PABS† 4.8 gm.	3,640	153.6	107.0	118.9	1.52	0.3	14.2	1.2	7.2		
	PABS 3.6 gm.	2,400	110.8	69.2	85.0	0.84	0.1	9.5	1.4	7.2	53.2	3.7
	PABS 3.6 gm.	2,710	111.5	68.7	130.6	1.17	0.2	11.6	1.6	6.8	49.7	4.2
	PABS 5.4 gm.	1,995	78.7	71.8	100.9	0.96	0.2	10.1	1.6	6.7	52.9	4.3
	PABS 5.4 gm.	2,095	85.0	44.6	75.4	0.77	0.1	8.5	1.8	7.2	52.2	9.2
	PABS 5.4 gm.	2,820	130.0	91.6	147.5	1.23	0.4	16.4	3.4	6.8	53.9	8.9
	Control	2,110	99.3	48.8	124.0	1.10	0.6	13.3	1.7	6.6	51.9	10.0
	Control	2,190	81.4	54.2	149.1	1.01	0.9	13.4	0.3	5.6	55.4	1.5
	Control	1,960	79.4	52.0	119.0	1.07	0.7	12.9	0.1	6.0	60.2	Trace
	Control										71.8	0.0
L. H.	Control	2,240	64.2	78.8	87.8	1.10	0.3	11.3	0.0	6.9		
	Control	2,170	53.9	80.7	75.0	0.96	0.3	10.6	0.0	6.8	60.1	0.0
	PABS 4.8 gm.	3,070	135.0	136.3	94.6	1.01	0.1	10.6	1.1	7.8		
	PABS 4.8 gm.	2,600	106.5	98.4	85.0	0.87	0.1	9.8	1.7	7.6	57.9	5.5
	PABS 4.8 gm.	2,040	140.5	110.0	115.0	0.63	0.1	11.0	2.2	7.5	49.9	6.8
	Control	1,555	126.4	77.2	119.6	0.70	0.4	11.7	1.1	7.0	46.8	7.0
	Control	1,415	49.5	45.9	93.7	0.92	0.6	12.0	0.3	6.0	46.8	2.3
	Control	1,515	37.5	65.6	112.9	1.33	0.9	13.5	0.1	5.8	54.2	1.6
	Control	2,065	46.5	73.1	96.1	1.38	0.8	14.0	Trace	6.3	61.5	Trace
	Control	2,280	67.6	76.6	108.7	1.37	0.6	12.8	0.0	6.5	62.8	0.0
J. M.‡	Control	1,753	33.8	31.1	51.8	0.76	0.13	5.73	0.00	6.5	57.2	0.2
	PABS 4.8 gm.	3,000	85.4	87.6	85.0	1.27	0.15	9.24	0.78	7.0		
	PABS 4.8 gm.	3,070	123.0	118.1	102.1	1.39	0.08	11.74	1.77	7.7	54.6	5.9
	PABS 4.8 gm.	2,665	110.0	67.2	103.6	1.13	0.09	9.22	1.68	7.4	51.2	9.2
	PABS 4.8 gm.	2,240	87.3	99.6	91.3	1.16	0.13	9.85	1.64	6.8	51.3	9.1
	PABS 4.8 gm.	3,163	82.8	73.4	111.4	1.55	0.24	14.39	2.95	6.6	53.8	7.4
	Control	1,885	51.9	36.2	61.4	0.73	0.24	7.17	1.16	7.2	50.9	7.7
	Control	2,510	76.5	51.1	113.2	1.37	0.41	11.23	0.64	6.0	55.3	2.4

* All chemical analyses on urine represent total quantities in the 24-hour period.

† PABS is an abbreviation for sulfanilamide (para-amino-benzene-sulfonamide).

‡ This patient was not in balance with his diet and the preliminary control period is obviously worthless, but the general trend agrees with the other two cases.

was noted. There was no appreciable change in the excretion of chloride, inorganic phosphate, or total nitrogen.

During sulfanilamide administration there was a fall of 13.5, 13.3 and 6.3 volumes per cent, respectively, in the CO_2-combining capacity of the plasma of the three subjects. Blood sulfanilamide concentrations rose to 10.0, 7.0 and 9.2 mgs. per cent, but there was no correlation between this rise and the drop in the CO_2-combining capacity (See Table I).

In both the subjects that were adequately followed the sulfanilamide was almost completely eliminated 48 hours after the last dose, and in none of the three subjects did daily examinations of freshly voided specimens reveal evidence of renal damage. Blood specimens were negative when examined spectroscopically for sulphemoglobin and methemoglobin, even though the subjects showed a slight blue discoloration. Malaise and nausea were noted. No cutaneous rash appeared. Fever was present in all subjects, reaching a maximum of 102° to 103°F.

Two normal dogs were placed on a constant metabolic regime, and studies similar to those described for the human subjects were made. When sulfanilamide was given by stomach tube in divided doses (total of about 0.5 gm./kg.) there was a slight but definite diuresis and an increased excretion of fixed base. The changes, however, were much smaller than those observed in the human subjects and the plasma CO_2-combining power was not altered. These results are not incompatible with those of Marshall, Cutting and Emerson, for these authors used considerably larger single doses in their dogs to obtain a drop in the CO_2-combining capacity.

SUMMARY

(1) In three normal human subjects, the administration of sulfanilamide was accompanied by a decrease in the CO_2-combining capacity of the plasma, a definite diuresis and an increase in the renal excretion of sodium and potassium.

(2) Two dogs showed slight losses of base through renal excretion but not enough to alter the CO_2-combining capacity of the plasma even though they received larger doses of sulfanilamide per kilogram of body weight than did the humans.

The authors are greatly indebted to Dr. Perrin H. Long, Dr. George Harrop and Dr. George Thorn for their invaluable assistance and suggestions.

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TWO CASES OF PELLAGRA TREATED WITH NICOTINIC ACID

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The purpose of this report is to record two additional cases of pellagra treated with nicotinic acid. Recent work (1, 4, 5, 6) has shown that the administration of this substance, perhaps the active pellagra-preventing principle, may effect a remission of many of the signs and symptoms of this disease.

REPORT OF CASES

Case 1: M. S., white female of 64 years, unmarried, entered the ward of the Union Memorial Hospital on December 29, 1937, complaining of weakness, loss of weight, sore tongue, and an eruption on the hands. Except for her diet, the past history was essentially unimportant. She was born in Baltimore and had lived there practically her entire life. She denied using alcohol, but admitted that her diet during the past two years had been almost devoid of vegetables, fruits and fresh meats. Roughly, her menu consisted of bread, butter, canned food such as potted ham, syrup and fat meat. She had lost about twenty pounds in weight during this period, but did not develop the dermatitis or sore tongue until two months before admission. About the middle of November she began to have a profuse diarrhea which averaged six to eight stools a day and persisted until just before entry into the ward. There were attacks of giddiness during which she would feel as if she were going to faint. Associated with these was a progressive generalized muscular weakness.

Physical examination: A poorly nourished woman of about 65 years. Temperature 98.2°, Pulse 80, Respirations 16. Blood pressure 120/82. Mentally apathetic and lethargic. There were no delusions. She seemed normally oriented as to time and place. Skin over the hands and wrists dry, coarse, scaling and deeply pigmented, with a well defined margin of demarcation between the affected and unaffected areas. Ulceration was present over the knuckles, while between the fingers and over the flexor surfaces of the wrists the skin was deeply fissured. There was moderate pigmentation and roughening of the skin over the feet and

elbows. The tongue was beefy red and painful, with atrophy of the papillae around the margins. Reflexes sluggish; there was moderate facial weakness of the peripheral type on the left. The remainder of the physical examination afforded no significant findings.

Laboratory studies: RBC 3.12 million; Hb. 85% (Sahli); WBC 4,600, PMN 67%. Urine showed a trace of albumin. Blood Wassermann: negative. Gastric analysis (Ewald meal) Free acid: 2.2° Total Acid: 4.0°.

TABLE I

Case 1

DATE	TREATMENT	CONDITION OF HANDS	TONGUE	MENTAL CONDITION	EKG
12/29/37	Admitted. Given diet deficient in the B group of vitamins				
1/ 2/38	Started on nicotinic acid 0.1 gm. q.d.	Ulcerated, scaling, rough and pigmented	Sore, red	Apathetic, sluggish	#1
1/ 2/38			Redness less marked		
1/ 3/38		Unchanged	Healed	Unchanged	
1/ 5/38		Unchanged		Unchanged	
1/ 7/38		Beginning healing of ulcers		Unchanged	
1/12/38	Nicotinic acid increased to 0.1 gm. t.i.d.	Skin softer, smoother, less pigmented		Less apathetic	
1/20/38		Ulcers healed		More alert	#2
1/25/38	Nicotinic acid discontinued	Skin essentially normal. Slight residual thickening over knuckles			
1/26/38	Placed on house diet with additional Vitamin B				

Electrocardiographic Note: No. 1, 1/1/38, Normal except for T waves less than 0.5 mm. in leads I and II. Number 2, 1/20/38, Normal. T I = 1 mm., T II = 2 mm.

The restoration to normal of small or iso-electric T waves in cases of pellagra responding to treatment has been previously noted (2, 4).

On January 1, 1938, the patient was placed on a diet deficient in the B group of vitamins. She received Irish potatoes without skins, bread, butter, bacon and fat meat but no milk, red meat, fruits or green vegetables. Nicotinic acid,[1] 0.1 gm., was given orally each day. Twelve days later the dosage was increased to 0.1 gm. three times a day.

Her course in the hospital is shown in Table I.

[1] Two different preparations of nicotinic acid were used in this case: that of the S. M. A. Corp. and that of the Eastman Kodak Company.

Comment: The rapid and complete healing of the glossitis was perhaps the most striking feature of the general improvement in this patient. However, the gradual replacement of the scaling, leathery, ulcerated skin by pinkish, smooth, delicate epithelium was very impressive. Improvement in the mental lethargy was definite, although the patient's mental status was still abnormal at the conclusion of treatment.

Case 2: G. S., a 47 year old negress, was admitted to the medical ward of the Johns Hopkins Hospital (No. 130,439) on February 14, 1938 with the complaint of "sore mouth and sore privates." Her past history was colored by repeated miscarriages, apparently due to syphilis. In 1934 her blood Wassermann test was found to be positive and antisyphilitic treatment was instituted. This she had taken somewhat irregularly over the next four years. This patient had visited the accident room on numerous occasions with various manifestations of acute and chronic alcoholism, but she denied the use of alcohol during the past three years. For six months prior to hospitalization she had eaten very little because of lack of funds. Her average daily food intake over this period consisted of ½ can of milk, 1 piece of fresh fruit, 1 bowl of oatmeal or rice, cocoa (made with water), and spinach (once a week). She lost 30 pounds in weight. Six weeks before admission to the hospital she had developed a vaginal discharge and painful sores in the groins and perineum, and for the last ten days had suffered from soreness of the mouth and tongue so severe that she could scarcely eat at all. One week before entry she first noted dry rough patches on the elbows, wrists, and hands. There was no diarrhea prior to admission.

Physical examination: A surly, apathetic colored woman who had obviously lost weight. Temperature 100.6°, Pulse 112, Respirations 24, Blood pressure 150/85. The skin showed symmetrical dry, thickened, hyperpigmented areas over both elbows and wrists and on the hands between the fingers, with a tendency to exfoliation at the latter site. The tongue was extremely red, especially about the margins, with some flattening of the papillae and a grayish-yellow exudate covering small marginal ulcers. The buccal mucosa was fiery red and ulcerated and the entire mouth was extremely sensitive. The examination of the heart, lungs, and abdomen revealed little abnormal other than the tachycardia and moderate abdominal distention. The perineum was red and sore with weeping ulcers; the vulval and vaginal mucosae were a bright red and exquisitely tender to the touch. There was a profuse foul vaginal discharge. No neurological abnormality was made out except absence of the ankle jerks.

Laboratory Studies: RBC 4,950,000, Hb. 109% (Sahli), WBC 14,000, differential formula normal. Blood Wassermann negative. Blood chemistry: NPN 24 mgm. %, serum chloride 84 m. eq., total protein 8.17 gm. % with A/G 45/55. The urine showed a trace of albumin and many leucocytes. Stool negative. X-ray of the chest showed normal heart and lungs. An electrocardiogram showed

sinus tachycardia; no other abnormality. Gastric analysis revealed no free HCl even after histamine stimulation. Vitamin A deficiency was demonstrated in that her dark-adaptation time was 9 minutes (normal 2–3 minutes) and the blood vitamin A level was 0 units (normal 1.5–3 units).[2]

Course: The course of this patient is depicted in Fig. 1. Immediately upon admission she was placed upon the Standard Basic Diet 2 of Ruffin and Smith (3)

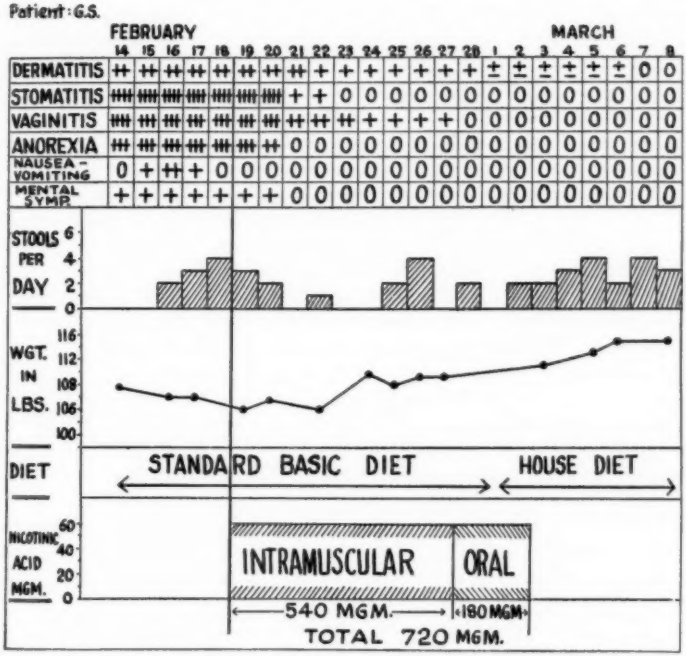

FIG. 1. CASE NO. 2. THE EFFECT OF NICOTINIC ACID ON THE SYMPTOMS AND SIGNS OF PELLAGRA

with two minor changes: the calcium gluconate was omitted and halibut liver oil (60 drops a day) was substituted for cod liver oil. She was kept on this diet alone for a 5-day control period during which time there was no improvement in any of the symptoms or signs of the disease. Indeed she ate rather poorly because of

[2] The authors are indebted to Dr. Hugh Josephs and Mr. Howard F. Conn for the vitamin A studies in Case 2.

complete anorexia and the soreness of her mouth. She developed a mild diarrhea and lost 4 pounds in weight. Her mental state grew steadily worse.

On February 19 therapy with nicotinic acid[3] was begun. For the next 9 days the patient was given daily intramuscular injections of 60 mgm. of nicotinic acid dissolved in sterile normal salt solution. The injections caused little local discomfort but were followed in a few minutes by mild generalized sensations of tingling and flushing. During the first two days of this treatment there was no apparent objective change in the lesions but she said that her mouth was less sore and her appetite better. On the third day of treatment the condition of the tongue and buccal mucosa showed remarkable improvement. The fiery red color had given way to a more normal pink and the ulcers showed evidence of healing. The mouth appeared perfectly normal 5 days after the first injection of nicotinic acid. Equally striking was the change in disposition that took place. The previous surliness and apathy were replaced by an exuberant euphoria. The patient laughed and joked with the doctors, and developed such a craving for food that she devoured her rather distasteful diet with relish and begged for more when she was through. At the same time the vaginal tenderness disappeared and the perineal lesions began to heal. The diarrhea ceased in spite of the increased food intake and she gained 5 pounds in the next 5 days. As might be expected, the skin lesions cleared more slowly; the rough thickened patches gradually loosened and fell off, leaving delicate normal skin beneath. On the 7th day after the start of treatment gastric analysis revealed 7° of free HCl after histamine.

The intramuscular injections of nicotinic acid were continued for nine days; for the next three days nicotinic acid was given by mouth, 60 mgm. a day. The medication was then discontinued and the diet changed from the pellagra-producing ration to the regular house diet. The patient gained 6 pounds more before she was discharged from the hospital on March 8, 23 days after admission and 18 days after the first dose of nicotinic acid. At the time of discharge she showed no residual stigmata of pellagra. The dark adaptation time had dropped from 9 minutes to 4½ minutes, presumably as the result of Vitamine A administration in the form of halibut liver oil.

Comment: This 47 year old colored woman with moderately severe pellagra was given a basic dietary ration for a control period of 5 days during which there were no signs of improvement. The diet was then supplemented with daily intramuscular injections of nicotinic acid. Within 48 hours of the institution of nicotinic acid therapy the mucosal lesions showed remarkable improvement and several days later had entirely cleared up. Coincident with the disappearance of the mucosal lesions there was a dramatic change for the better in the mood and

[3] The Eastman Kodak Company preparation was used.

disposition of the patient. The skin lesions healed steadily but slowly. The patient received in all 720 mgm. of nicotinic acid, 540 mgm. by intramuscular injection and 180 mgm. by mouth.

DISCUSSION

We have presented two cases of moderately severe pellagra treated with nicotinic acid, which was given orally in the first case and by intramuscular injection in the second. Prompt healing of all of the lesions of the disease took place in both instances. These observations are in accord with those made at several other clinics in this country within the past six months.

It seems desirable to emphasize the economy of treatment with nicotinic acid in pellagra as exemplified by the two cases we have reported. The first patient received in all 4.9 grams of nicotinic acid by mouth over a period of 23 days. The total cost of the nicotinic acid used amounted to about 70 cents, making the cost of the daily dose about 3 cents. The second patient was given 720 milligrams of nicotinic acid in all over a 12-day period; the cost of the drug used was about 10 cents, or less than one cent per day. The extremely low cost of the "specific" therapy in these two cases represents a tremendous reduction in the expense of the treatment of pellagra, which formerly ran as high as three to five dollars a day for the amount of liver extract, yeast and wheat germ required to bring about equally rapid improvement in cases of this type.

More recently at the Johns Hopkins Hospital, we have had occasion to treat two more cases of pellagra in colored women (No. 133,540 and No. 135,104) and two cases of post-operative glossitis (No. 130,400, a 25 year old white woman with a pyonephrosis, and No. 135,040, a 61 year old colored man after a gastro-enterostomy) with nicotinic acid. Since both of the cases of pellagra were complicated by severe infections (pyelitis in one, extensive pulmonary tuberculosis in the other), the diet was not restricted as in the cases reported in detail above. However, despite the infection the healing of the mucosal lesions under oral therapy with nicotinic acid occurred within several days in each of these cases. The improvement was considered to be more rapid than was to be expected from simple dietary measures alone.

Both instances of postoperative glossitis (to all intents and purposes

early pellagrous stomatitis) occurred in patients who had vomited so frequently before and after operation that for several weeks their chief nourishment had been derived from intravenous glucose. Within one week after operation each of these patients developed a very sore red tongue with superficial ulcers covered with yellow purulent exudate along the margins. Anorexia and marked asthenia were prominent features. Nicotinic acid, 60 mgm. a day, was given by intramuscular injection in each case, and within 72 hours the glossitis had cleared up completely. The soreness of the tongue disappeared, the appetite increased, and there was a pronounced change for the better in the general condition of the patient. Such observations suggest that nicotinic acid may prove useful both therapeutically and prophylactically in the treatment of patients with persistent vomiting or anorexia and in postoperative states when the intake of food and vitamins by mouth must be restricted for a considerable period, as for example after operations upon the stomach.

SUMMARY AND CONCLUSIONS

Two cases of pellagra are reported in which nicotinic acid appeared to effect remissions as regards the lesions of the skin and mucous membranes. The general strength and mental status were much improved in both patients.

In the electrocardiogram of the first patient, T waves of small amplitude returned to normal. In the second case free hydrochloric acid reappeared in the gastric juice within one week after the therapy.

The low cost of the treatment of pellagra with nicotinic acid in contrast to earlier forms of therapy is stressed.

The intramuscular use of nicotinic acid, both therapeutically and prophylactically, is advocated for the treatment of patients whose intake of food is by necessity very limited. Such a situation commonly arises in cases of persistent vomiting and anorexia or in postoperative states following major surgical procedures involving the gastro-intestinal tract.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

On Thought in Medicine (Das Denken in der Medizin). By HERMANN VON HELMHOLTZ. An Address delivered August 2, 1877, on the Anniversary of the Foundation of the Institute for the Education of Army Surgeons. *Introduction by Arno B. Luckhardt.* 27 pp. 75¢. *The Johns Hopkins Press, Baltimore, 1938.*

In this address Helmholtz sketches the deplorable state of medical thought in Germany as it was when he was a student thirty-five years before, in the period when the minds of most medical men were still saturated with paralyzing dogmas built upon unfounded hypotheses. At that time, Helmholtz tells us, to feel the pulse was regarded as the most important means of clinical investigation, and there were still clinicians who clung to the view that auscultation and percussion debased the patient by treating him as though he were a mere machine. He recalls a celebrated colleague who would never even try the ophthalmoscope because he regarded it as dangerous to admit crude light into diseased eyes. It was a period in which many of the influential and respected leaders in medicine found it impossible to adjust themselves to the rapidly developing critical scientific attitude that was spreading throughout all branches of medicine. The new period of eager search for fact by observation and experiment was, however, soon in ascendency, and it developed with such amazing fruitfulness that within thirty-five years the metaphysical concepts and attitudes which Helmholtz had encountered as a student had already become history unfamiliar to the younger generation.

While this address is interesting historically, it is difficult for the reviewer to find in it the appositeness to present day conditions upon which Dr. Luckhardt insists in his introduction. "Then, as now," he writes, "ideas and cogitation were emphasized beyond all reasonable bounds and to the virtual exclusion of fact finding by observation and thoughtful experimentation." This is a surprisingly excoriating indictment of the present. Theorizing writing-desk scientists are probably as exceptional today as were critical observers and experimenters in the "dark metaphysical period." Never in the history of science have ideas been held up to so merciless (or merciful) a scrutiny by observation and experiment as at the present time.

Nor can the reviewer find in this address anything bearing upon the problems in medical education which have recently been engaging the attention of various faculties, and for which Dr. Luckhardt regards this discourse as providing the

solution. Indeed, one wonders why, if the purpose of this reprint was to present Helmholtz's views on education, his more pertinent rectorate address, "Über die akademische Freiheit der deutschen Universitäten," written in the same year, was not reprinted instead.

A. R. R.

Handbook of Orthopaedic Surgery. By ALFRED RIVES SHANDS, JR., B.A., M.D., in collaboration with RICHARD BEVERLY RANEY, B.A., M.D. 593 pp. Illus. $5.00. (*The C. V. Mosby Company, St. Louis, 1937.*)

This is a textbook for the use of the medical student and the general practitioner. It is not an encyclopaedia covering the whole field of orthopaedic surgery, nor is it a monograph in which the author stresses his special interests or researches. It is a well-rounded survey of the subjects that constitute orthopaedic surgery (with the exclusion of fresh fractures, plaster technique, and orthopaedic appliances), and affords a framework for the student's studies in this field. To facilitate more intensive investigation there is supplied a well chosen bibliography. There has been a rather interesting handling of the matter of illustrations. In place of reproductions of X-rays and photographs so frequently used, the artist has supplied pen and ink drawings. Not only does this procedure help in keeping down the price of the book but it permits the artist to emphasize any points which are of particular interest.

A textbook of this type fills an existing need in the teaching of orthopaedics. The scope of this branch of surgery has been so amplified in recent years and the reports of fundamental investigations, new concepts, and modern therapeutic procedures have been so numerous, that textbooks have been becoming progressively larger and heavier. On the other hand, the amount of time allotted by medical schools for the teaching of orthopaedics of necessity has been limited. This limitation is applicable not only to the number of lecture hours but also to the time available for home study, and textbooks and teacher must therefore be selective in the material presented to their students. The authors of the Handbook have been quite successful in presenting the highlights of the various subjects. The method that they have chosen to present this material conforms with the teaching schedule that is most generally used in this country. The material is divided into 24 chapters, so chosen that they will fit in with the plans of the Committee of Undergraduate Instruction in Orthopaedic Surgery for a twenty-four class period during the school year. Although this textbook is recommended particularly for the use of students and general practitioners, it has a very definite value in the library of the orthopaedic specialist.

I. W. N.

Short Years. The Life and Letters of John Bruce MacCallum, M.D., 1876–1906. By ARCHIBALD MALLOCH. 343 pp. Illus. $3.50. (*Normandie House, Chicago, 1938.*)

This unique biography portrays a gifted young scientist who before the age of

30 published a series of brilliant discoveries in microscopic anatomy, embryology and physiology, in spite of a devastating illness during the last six years of his life that would have downed any ordinary mortal long before the end.

The author with rare delicacy and understanding has woven into the story many of MacCallum's letters to his family and especially to two girl friends, letters that reveal phases of his inner life not familiar even to close friends. The letters give charm and an unusual quality to the biography, almost converting it into an autobiography where the author becomes editor. They are the outstanding feature of the book and expose a rare personality of charm, insight and thoughtfulness. They help one to understand how he accomplished so much in such a short time. Their literary and artistic value will appeal not only to young medical men and scientists but to the general reader as well.

Canadian born and a graduate of the University of Toronto at 20, MacCallum's undergraduate days held inklings of literary and scientific aims. During his four student years (1896–1900) at the Johns Hopkins Medical School, inspired by Mall and Barker, his genius for scientific investigation developed with surprising rapidity; five noteworthy researches were completed, dealing with normal and pathological heart muscle, with skeletal muscle and with the muscular architecture of the heart. His last years at the University of California with J. Loeb were especially productive in spite of failing health and very short hours at the laboratory.

MacCallum had the unusual capacity of visualizing the essence of the problem before him and of arriving at a solution where others floundered. His artistic ability enabled him to express clearly in words and in drawings his results.

The author has done a fine job and the publishers have set up an attractive book.

W. H. L.

Biological and Clinical Chemistry. By MATTHEW STEEL. 770 pp. Illus. $8.00. (*Lea & Febiger, Philadelphia, 1937.*)

This is an attempt "to blend theoretical and practical biochemistry with chemical pathology and clinico-chemical methods." There is an obvious advantage to the beginner in having available in a single text much material which is usually scattered and often difficult to assemble and to correlate. The interspersion of laboratory experiment and clinical application with text-book development of the chemistry and theory of the science is novel and is worthy of serious consideration as a pedagogical approach to the subject. In a general way, the text attains a modicum of success; critically considered, however, the goal falls short of achievement for several reasons.

Advances in biochemistry are being made so rapidly that a comprehensive text by a single author is necessarily outmoded when published. Furthermore, the various divisions or chapters on the subject are so highly specialized and so widely different in character as to make it impossible for any one person to speak authoritatively on more than a small part of the whole. It is not surprising,

therefore, that in addition to the many typographical errors which often appear in first editions there are errors of a more serious sort which reflect the author's lack of first-hand knowledge of his subject. Particularly weak are the chapters on the quantitative chemical analysis of the blood, the biophysics of blood and certain discussions of the clinical significance of faulty metabolism.

M. V. B.

Surgical Diseases of the Mouth and Jaws. By EARL CALVIN PADGETT. 807 pp. Illus. $10.00. (*W. B. Saunders Company, Philadelphia & London, 1938.*)

This very complete text on the surgical diseases of the mouth and jaws contains a good description of the anatomy and to a less extent the physiology of these regions. It then takes up, step by step, the pathological conditions found. The description of these lesions often includes some historical facts dealing with past therapy as well as the pathology, etiology, diagnosis and treatment. Care has been taken not to be too dogmatic on any disputed points of etiology or therapy, and where such points exist, the opposing views are presented. Indeed the sole criticism might well be that in several instances these presentations were somewhat involved and so impartial that one was left in doubt as to the author's own belief. The latter part of the book contains chapters on the plastic surgery of this region as well as descriptions of various prosthetic and dental devices. There is also a chapter devoted to the irradiation treatment of this area, discussing methods of exposure, dosage, theories, and results. The concluding chapter presents the different methods of anaesthesia for operative attack upon these lesions.

The book is well written, adequately illustrated and conveniently arranged so that related conditions and their differential diagnosis can be compared. The sections on therapy are quite specific and detailed.

We have here a very complete volume on its subject. It contains information which should be useful to dentists as well as physicians. For dental or oral surgeons, as well as general surgeons, it should be most valuable for reference. To the general practitioner of medicine and the medical student it will give a familiarity with this region to which they are, too often, strangers. The plastic surgeon will be better served by other volumes on his subject, but the general practitioner of dentistry should find much that will help in the early recognition of lesions seen during the dental care of his patients.

R. T. S.

A Textbook of Ophthalmology. By SANFORD R. GIFFORD. 492 pp. Illus. $4.00. (*W. B. Saunders Company, Philadelphia 1938.*)

This textbook, intended for medical students and general practitioners, is issued in the same general format as an older treatise on diseases of the eye, which has enjoyed reasonable popularity for many years. Dr. Gifford follows the conventional method of presenting his subject, dividing the book into twenty-one chapters, dealing with methods of examination, errors of refraction, diseases of

the eyelids, conjunctiva, cornea, sclera, uveal tract, etc. A remarkable amount of material has been crowded into a relatively small space, with the natural result that the descriptions of different disease entities and discussions are necessarily brief, and at times somewhat inadequate. The subject matter is, however, remarkably up to date and is clearly and concisely presented. The illustrations are in many instances new, and when not original have been borrowed from more recent textbooks, with the result that there is a refreshing absence of the time-worn and antiquated illustrations which have almost become standard in so many ophthalmological textbooks. Photographs, which are freely used, are excellent and well reproduced. The color plates are not so uniformly good.

All in all, this new textbook, while far removed from the class of reference work, to which it does not aspire, is a distinct improvement on other books of like size, and can well be recommended to medical students.

A. C. W.

Mental Therapy. Studies in Fifty Cases. By LOUIS S. LONDON. 774 pp. in 2 volumes. $12.50. (*Covici-Friede, New York, 1937.*)

This is an attempt to present the therapy of neuroses, psychoses, and sexual perversions by psychoanalytic methods.

In the preface the author states "the book is presented as a reference to the psychologist, neurologist, psychiatrist and the general practitioner, who will find the cases presented of decided value in their practice." To speak frankly, the reviewer has not read the entire book, but from samples taken throughout he feels privileged to doubt their "decided value." The book is full of psychoanalytic dogma and doctrine, and from that standpoint it gives a lucid enough presentation of the material. The excessive weighting of the psychosexual material in every case is not to be wondered at in view of the bias on which the book is written, but one cannot help but feel that many psychopathologic and therapeutic items have been left completely untouched in the final evaluation. Furthermore, in reading some of the cases, for example, case 20, "Analysis of a Case of Satyriasis," the reviewer fails to see the therapeutic implications of the presentation, and what is to be gained by a chronological account or reiteration of sexual exploits without any attempt at a constructive formulation is hard to see.

It may be that psychoanalysts will find these volumes of some importance, but from the standpoint of a psychiatrist in active contact with patients it appears to this reviewer to be of very doubtful value.

W. M.

UNIV. OF MICH.

ADDRESS ON THE OCCASION OF THE PRESENTATION OF A PORTRAIT OF DR. EDWARDS A. PARK TO THE JOHNS HOPKINS UNIVERSITY, FEBRUARY 22, 1938

RUSTIN McINTOSH

Mr. Provost:

Dr. Edwards A. Park, since 1927 professor of pediatrics in this university and pediatrician-in-chief of the Johns Hopkins Hospital, has many warm friends and devoted admirers. It is my great privilege to speak for them on this occasion.

I shall not attempt to measure for you the scope of our admiration and affection for Dr. Park. I shall try merely to tell you, as directly as I can, what he means to medicine and, in particular, to pediatrics today. In the first place, as a physician he is a master in both the science and the art of his profession. That is to say, he possesses to an unusual degree the ability to draw upon his own observations and upon information accumulated from the experiences of others in various branches of medicine and, by using the inspirational process of selection which characterizes the clinical art, to pick out and apply that which will mean the most to the individual patient. Then, as a teacher his influence has made itself felt far beyond the walls of the immediate classroom. Thirdly, as an investigator in biological science he has added greatly to the sum of exact knowledge, especially in regard to the behavior of osseous tissue under normal and abnormal conditions. Many of you realize, no doubt, that rickets, a disease affecting the skeletal system of infants, was less than a generation ago so prevalent in urban populations as to involve nine out of every ten children living in crowded homes. Rickets has by no means been eradicated; but our knowledge of its treatment, and particularly of its prevention, has advanced to the point where, under suitable control of environmental factors, it can be made actually a rare disease. Dr. Park's contributions to this accomplishment are familiar to pediatricians the world over. Finally, and I shall only allude to it, there is his influence as a man—as a philosopher, if you will. I refer to the

effect he has had on those who have enjoyed the privilege of working in intimate association with him, his influence in showing them by his modest example what true values are.

It is Dr. Park's colleague, Dr. L. Emmett Holt, who has made it possible for us, for the friends whom I am happy to represent here, to achieve concerted action in bringing to the university a gift which we trust is worthy and which affords us the special satisfaction of signifying in some degree our personal devotion to the subject of this portrait. The artist, Mr. Paul Trebilcock, of New York, has produced a work of which we can well be proud.

It gives me great pleasure, Mr. Provost, on behalf of Dr. Park's friends, to present this portrait to the University.

THE CLINICAL AND PATHOLOGICAL FINDINGS IN INTERAURICULAR SEPTAL DEFECTS

A Report of Four Cases

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INTRODUCTION

The importance of the congential malformation of the heart which results from a gross defect in the foramen ovale, *i.e.*, a patent ostium secundum, is not generally recognized. Although this malformation is compatible with relative longevity, it is among the commonest of the cardiac malformations which in adult life bring the patient to the doctor. Not infrequently the patient is considered to have rheumatic heart disease and the nature of the condition is not recognized until autopsy. The mistake in diagnosis is made in part because of the lack of familiarity with the various clinical manifestations and in part because of the frequency with which the condition is complicated by valvular lesions, either congenital or acquired. It is the rule rather than the exception to find some lesion of the mitral or aortic valves combined with an interatrial septal defect (39). Although not yet conclusively proved, it seems probable that strain on the left side of the heart is an essential factor for the development of the clinical and pathological changes which are characteristic of this malformation. These characteristics are the great dilatation and hypertrophy of the right side of the heart, the enlargement of the pulmonary artery and its branches and the relatively small left ventricle and aorta.

Roesler (39), in his detailed analysis of 61 cases, found that more than three-quarters of them were complicated by valvular lesions,

most often of the mitral valve. Among the fifty-two cases in which the valves were mentioned only five had normal valves. In three of these cases (1, 32, 48) there was presumptive evidence of a systemic hypertension; in the other two (4, 22) no mention was made of the blood pressure or of the condition of the kidneys at autopsy.

In 1860 Peacock (36) described a heart which had a widely patent foramen ovale and a thickened mitral valve. Five years later Martineau (29) reported the first case of an interauricular septal defect associated with a mitral stenosis in which both the clinical and pathological findings were given. Following his paper a succession of reports have appeared in the literature. In 1916 Lutembacher (27) reported a case of a woman with a patent foramen ovale, a mitral stenosis and a greatly dilated pulmonary artery[1]. He expressed the opinion that the defect in the interauricular septum relieved the strain of the mitral stenosis and thereby increased the expectancy of life.

Assman (5) in 1928 and Dressler and Roesler (11) in 1930 described x-ray findings which they believed to be characteristic of this condition, namely, great right-sided enlargement of the heart, a prominent pulmonary conus, a small aortic knob and increased hilar shadows.

In 1933 McGinn and White (30) reported another case and reviewed the clinical as well as the x-ray findings in cases of interauricular septal defects associated with mitral stenosis. They believed that the diagnosis could be made from a fairly typical roentgenogram and suggestive clinical signs.

Since the reviews of Roesler (39) and McGinn and White (30) several single case reports have been published. Instances of an open foramen ovale associated with mitral stenosis have been described by Mitchell and Bauer (32), Jerofejeff (21), Gibson and Roos (16), Joules (24), Cesari (7), McLeod (31), Lutembacher (28), Sailer (41) and Cassio and Arana (6). In two of the recent cases, one of those reported by Gibson and Roos and one by Tarnower and Woodruff (45), a widely patent foramen ovale was the only anatomical abnormality. In the latter case, however, the patient suffered from hypertension and in the former no mention was made of the blood

[1] Since his report this combination of lesions has been known as "Lutembacher's syndrome."

pressure or of other abnormalities which might have increased the pressure in the systemic circulation. All the evidence so far adduced indicates that left-sided cardiac strain may be an essential feature.

During the last few years four examples with gross defects in the interauricular septum have been seen at the Johns Hopkins Hospital. In one case the patient was thought to suffer from rheumatic heart disease; the others were recognized as having a congenital malformation of the heart; only in one was the diagnosis made clinically. Notwithstanding the variation in diagnosis, these cases illustrate certain features by which the clinical diagnosis can be established; the purpose of this report is to review these features.

REPORT OF CASES

Case 1. C. C. (M.B.V. No. 53499) A white man of 47 years—an electrician—was first seen at the Johns Hopkins Hospital in 1915 at the age of 37.

Past history revealed that he had had severe tonsillitis when 15 years of age; that he was subject to frequent colds and sore throat and had had two attacks of "dry pleurisy".

Present complaint was shortness of breath of six years' duration. He also complained of paroxysms of dyspnea and volunteered the information that during these attacks his heart beat faster than normal.

Physical examination showed the heart to be of normal size and only a short systolic murmur was audible at the apex.[2] The history of attacks of dyspnea and palpitation which started and ended abruptly suggested that he suffered from paroxysmal tachycardia. The electrocardiogram showed a moderate right axis deviation and a bundle branch block. No record was obtained during an attack.

Course of illness: The attacks of palpitation persisted and the dyspnea gradually increased in intensity. He was not seen until June 1924. At that time his heart was enlarged; the first sound was abrupt and there was a gallop rhythm but no murmurs were heard. An X-ray showed the heart to be much enlarged and a note was made that the patient should be fluoroscoped "to rule out aneurysm". A year later he again returned suffering from congestive heart failure and was admitted to the ward.

Physical examination: The patient showed all of the characteristic features of severe congestive failure. There was a moderate degree of cyanosis but no clubbing of the fingers or toes. A deformity of the chest was noted which was described as a depression on the right side and a bulging on the left; this was considered to be the result of old rickets. The *heart* was greatly enlarged. The rate

[2] No X-ray was taken.

was rapid, 100 per minute; there were numerous extrasystoles. The first sound at the apex was almost entirely replaced by a rough systolic murmur which was transmitted to the axilla; the murmur was also quite intense in the second and third interspaces to the left of the sternum. A loud diastolic rumble was heard at the apex. The pulmonic second sound was accentuated. The blood pressure was 142/110 mm. of mercury.

Laboratory examination showed the following significant positive findings: The red blood count was 5,792,000. The hemoglobin was 90 per cent. The teleoroentgenogram showed the heart to extend 6 cm. to the right and 11 cm. to the left of the mid-line. There was no change in the electrocardiogram. The Wassermann reaction of the blood was negative.

Course in the hospital: The patient grew steadily worse; finally, he developed a severe tachycardia, became comatose and died.

Clinical diagnosis: Rheumatic heart disease, mitral stenosis and insufficiency, cardiac failure, and a history of paroxysmal tachycardia.

Autopsy No. 8797, performed by Dr. H. A. McCordock. The main interest centered in the heart. There was a deformity of the anterior thoracic wall in the region of the precordium. The pericardial cavity was free from excess fluid and its surfaces were smooth. The heart was enlarged; it weighed 620 gm. The right auricle was dilated. The foramen ovale was widely patent; the diameter of the opening measured 3 cm. The tricuspid valve leaflets were normal but the tricuspid ring was dilated, measuring 17 cm. in circumference. The cavity of the right ventricle was large; its wall was 1 cm. in thickness. There was a scar in the wall of the right ventricle, 2 cm. beneath the pulmonary ring to which a thrombus was attached. This thrombus extended up into the pulmonary artery. The pulmonary valves were delicate; the pulmonary ring measured 9 cm. in circumference. There were sclerotic plaques scattered throughout the larger branches of the pulmonary artery. The ductus arteriosus was closed as was the interventricular septum. The left auricle was smaller than the right. The mitral ring was 8 cm. in circumference. The mitral valve was thickened and shortened and so were the chordae tendineae, causing definite stenosis and insufficiency of the mitral valve. The left ventricular wall was 1.5 cm. in thickness and its cavity was small in comparison with the right. The aorta above the ring measured 4.5 cm. in circumference, while at the arch its circumference was 4.0 cm. and in the abdominal portion it was 3.75 cm. There was no arteriosclerosis. There were infarcts in the lower lobes of both lungs.

Microscopically, the *myocardium* showed a diffuse scarring; the scars were both fine and coarse. The larger scars covered a whole low-power field and in such places only a few intact muscle fibers remained. Some of the scars were perivascular. In general, however, the fibrosis resembled that which is seen in occlusion of many smaller coronary vessels, and in the lumen of one such vessel thrombus material was found. A thrombus was likewise found attached to the endocardium. The wall of the *pulmonary artery* showed intimal thickening but no cellular infiltration. The pulmonary artery, itself, contained both old and

fresh thrombi. Some of the smaller vessels of the pulmonary artery also showed definite, though not widespread, intimal thickening. There was, in addition, moderate emphysema and a good deal of coal pigment in the lungs. There was no arteriolosclerosis in any of the organs.

Anatomical diagnosis: Patent foramen ovale. Narrowing of aorta. Mitral stenosis and insufficiency. Cardiac hypertrophy and dilatation. Myocardial scarring. Mural thrombi in the right ventricle; thrombi attached to the pulmonary arterial wall. Infarcts in the lungs. Chronic passive congestion.

Case 2. G. T. (H.L.H. No. 62239; Unit No. 54572) A white boy of 8¾ years was first brought to the Harriet Lane Home in March 1929 with the complaint of pain in the back and swelling of the face.

Past history: The birth was normal (weight 8 pounds). No cyanosis was noted. Heart trouble was first discovered on routine examination at 5 years of age but at that time it caused no concern.

Present illness dated from an accident two weeks previously when a playmate jumped on his back and hurt it. Ten days later, *i.e.*, five days before admission, his face became swollen.

Physical examination on admission showed widespread evidence of congestive failure.

The *chest* showed a marked precordial bulge. The *heart* was greatly enlarged. The rate was slow—64 per minute. The blood pressure was 115/? (diastolic not obtained). The apex beat was visible in the fifth interspace beyond the midclavicular line. There was a pronounced systolic thrill in the second and third left interspaces. On auscultation there was a systolic murmur which was loudest in the second and third left interspaces. In addition, there was a systolic murmur at the apex, which was transmitted to the axilla and the angle of the left scapula, and an inconstant third heart sound. The pulmonic second sound was markedly accentuated. The extremities showed slight acrocyanosis but no clubbing.

Laboratory examination showed the following significant findings: The *teleoroentgenogram* showed the heart to be enlarged both to the right and the left. The *electrocardiogram* showed complete heart block (the auricular rate was 75 and the ventricular rate was 70) and a right axis deviation and notching of the QRS complex (Fig. 1). The *Wassermann* reaction of the blood was negative.

Course in the hospital: On rest in bed the boy showed great symptomatic improvement. Shortly after admission he developed a pronounced hematuria which persisted for many months.[3] In August 1929 after a tonsillectomy the boy was discharged from the hospital with the diagnosis of a congenital malformation of the heart and congenital complete heart block.

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[3] In spite of extensive laboratory studies the cause of the hematuria was never satisfactorily explained. The kidney function was rechecked in September 1931 and again in May 1932, and was essentially normal.

Course in Cardiac Clinic: During the winter of 1929–30 the respirations remained rapid (40 per minute) and both the complete heart block and the hematuria persisted. In December 1930, two months after his previous dispensary visit and twenty months after first coming under observation, although the heart

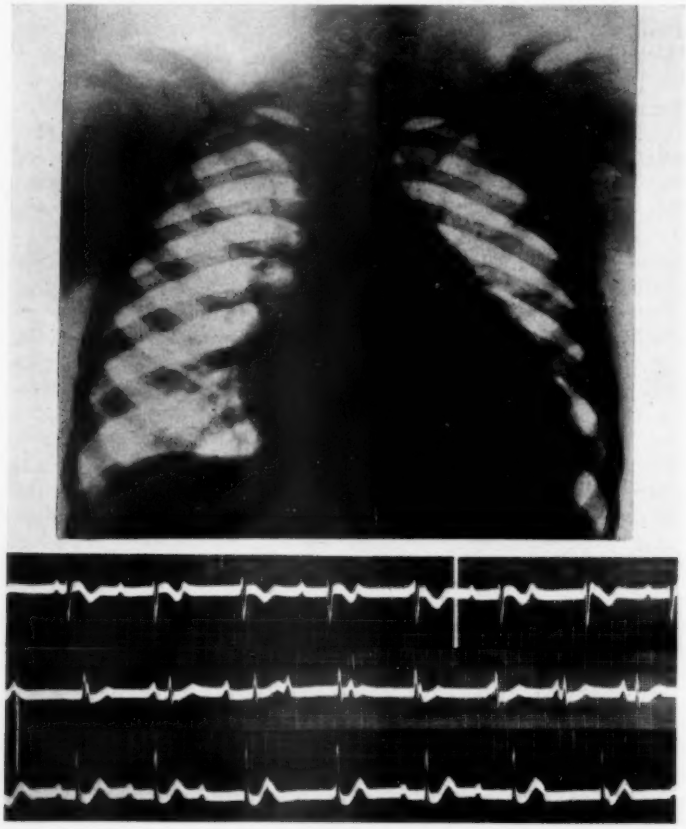

FIG. 1. X-RAY OF THE CHEST AND ELECTROCARDIOGRAM FROM CASE 2

showed no changes on percussion or auscultation, there was a well marked sinus arrhythmia. An electrocardiogram taken at this time showed a normal sinus mechanism with a P-R interval of 0.40 of a second, a QRS interval of 0.08 to 0.10 of a second, marked sinus arrhythmia with frequent periods of ventricular escape, and a right axis deviation.

As the patient became better, his attendance at clinic became more irregular. His last visit prior to his terminal illness was in May 1932. *Fluoroscopic examination* at that time showed marked enlargement of both the right auricle and the right ventricle. There was a conspicuous pulsation in the region of the pulmonary conus. It was not clear whether this pulsation was in the pulmonary conus of the right ventricle or in a dilated pulmonary artery.

On March 26, 1934 he was brought to the hospital with the complaint of a migratory polyarthritis of three days' duration. The heart again showed complete dissociation; no change was noted in the murmurs. He was sent home to bed on salicylates. Four days later he suddenly had a convulsion. When seen by the physician, the boy was intensely cyanotic, severely decompensated and suffering from pulmonary edema. He was immediately admitted to the hospital.

During *this admission* a diastolic murmur was heard for the first time along the left sternal border. Otherwise the findings in the heart remained essentially the same. He appeared to be improving rapidly when two weeks later he suddenly had a second attack of unconsciousness. The following day he had a third convulsion during which he died.

Clinical impression: Congenital heart disease with a superimposed rheumatic infection with mitral and aortic insufficiency; complete heart block with Stokes-Adams syndrome and shunt reversal. The congenital malformation was thought to be either a patent ductus arteriosus without a typical machinery murmur or an interauricular septal defect.

Autopsy No. 13675, performed by Dr. S. S. Blackman. The main interest centered about the heart. The chest bulged in the precordial region. The *heart* was large; it weighed 500 gm. The right auricle and ventricle were greatly dilated. The great veins entered the auricle in a normal fashion. The foramen ovale was 2 cm. in diameter and was not covered by a valve (Fig. 2). The tricuspid leaflets were a little thickened but the chordae tendineae were delicate. The tricuspid ring was dilated, measuring 12 cm. in circumference. The right ventricular wall measured 5 mm. in width; the cavity was large. The pulmonary ring measured 6 cm. in circumference. The pulmonary leaflets were a little thickened along their margins. The artery above them was 6.4 cm. in circumference. Above the left anterior pulmonary cusp there was a thickened irregular area in the wall about 2 cm. in diameter where the surface was wrinkled and rather translucent. The ductus arteriosus was closed. The interventricular septum was normal. The left auricle was smaller than the right and its wall was not as thick; the endocardium was smooth. Along the line of closure of the mitral valve there were warty nodules but the leaflets were not greatly thickened; the chordae tendineae were normal. The mitral ring was 6.2 cm. in circumference. There was some insufficiency but no stenosis of the mitral valve. Attached to the mitral valve at the junction of the anterior and posterior segments there was a thrombus (1 cm. in diameter) which projected into the orifice. The left ventricular wall measured 1.3 cm. in width. The aortic cusps were thickened with cord-like margins, producing a definite insufficiency. There were firm translucent vegetations along the line of closure. The aortic ring measured 5.5 cm. in circum-

ference and the aorta above was 5.8 cm. The thoracic portion was 3.5 cm. and the abdominal aorta was 2.4 cm. in circumference. The coronary arteries were normal. Both *lungs* showed patches of lobular pneumonia. The *liver* and *spleen* were enlarged and showed chronic passive congestion. The *kidneys* appeared to be normal.

Microscopically, in the *myocardium*, particularly that of the right ventricle, around several of the vessels there were collections of cells such as are usually seen

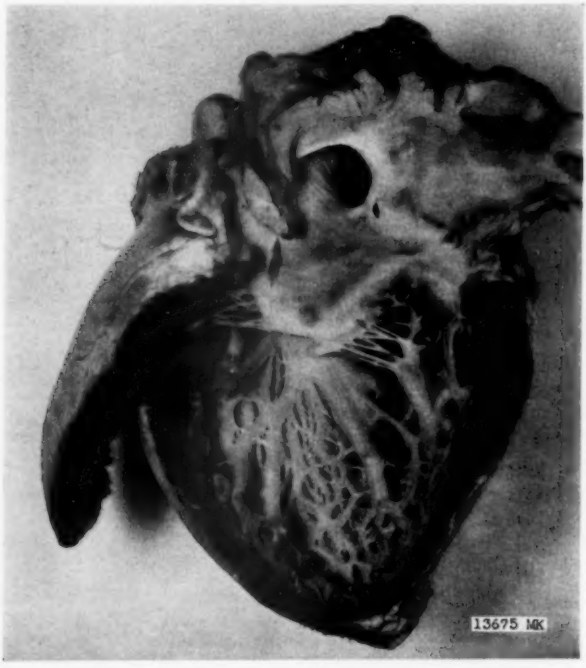

FIG. 2. SHOWS THE PATENT FORAMEN OVALE FOUND IN CASE 2

in Aschoff bodies. In the left ventricle there was distinct perivascular scarring and some perivascular round cell infiltration, but there were no fresh Aschoff bodies. There were rather large scars in the interventricular septum. The pulmonary artery showed a marked change. This consisted of a thickening of the adventitia which contained numerous small vessels surrounded by lymphocytes. In the media there were scars which contained blood vessels extending in from the adventitia; these vessels were likewise surrounded by mononuclear cells.

The intima was irregularly thickened by scar tissue and infiltrated with cells.[4] The pulmonary vessels of both large and small calibre, as well as the arteries and arterioles throughout the body, showed no sclerosis. The other *organs* showed only chronic passive congestion. The *kidneys* showed nothing to account for the previous acute hematuria.

Anatomical diagnosis: Congenital malformation of the heart—large patent foramen ovale; rheumatic endocarditis; scarring of the tricuspid, pulmonary, mitral and aortic valves; vegetations on the mitral and aortic valves; organized thrombus on the mitral valve; rheumatic lesion in the pulmonary artery; mitral, aortic and pulmonary insufficiency; cardiac hypertrophy and dilatation, especially of the right auricle and ventricle; scarring of the membranous interventricular septum; localized scar in the endocardium of the left ventricle beneath the aortic valve. Chronic passive congestion.

Case No. 3.[5] E. M. (J. H. H. Unit No. 66746) A colored woman of 47 years who first came to the hospital in August 1935 with the complaint that for the past eight months she had suffered from progressive increasing fatigue, with "misery" in her chest and attacks of dyspnea.

Past history: Birth was normal. No cyanosis had ever been noted. The patient stated that she had always become fatigued more easily than the other children. She had suffered from "sore knees" at the age of 15 and again at 24 which she attributed to housework and scrubbing. She had had nine full-term pregnancies and one miscarriage. During each pregnancy she had suffered from "swelling of her legs and thighs" and two or three times she had been "swollen all over."

Physical examination showed an underdeveloped and poorly nourished negress. There was slight arteriolosclerosis of the retinal arteries. A marked tracheal tug was present and a pulsation was visible in the suprasternal notch. A precordial bulge was noted and on each side of the chest there was an accessory nipple. The *heart* was enormously enlarged; the apex lay in the sixth left interspace. A prominent impulse was noted in the second and third interspaces on the left. There was a harsh systolic murmur with an accompanying thrill which was loudest in the third left interspace. This murmur was audible in the vessels of the neck and in the left interscapular region. A short, high-pitched diastolic murmur was heard in the pulmonic area. The pulmonic second sound was snapping. No diastolic murmur was audible in the mitral area. The pulses were of small volume. The blood pressure was 115/76 mm. A few moist râles were heard at the bases of the lungs. There was no cyanosis or clubbing of the fingers.

Laboratory examination: The hemoglobin was 96 per cent and the red cell

[4] These changes resemble those described by Kugel (26) in acute rheumatic fever.

[5] This case has previously been reported by Dr. S. W. Jennes (20).

count was 5,100,000. The teleoroentgenogram showed a greatly enlarged heart with an enormously dilated pulmonary conus and a large hilar shadow on the right. On fluoroscopy the dense shadows on either side of the heart were seen to pulsate and in the kymogram they showed an arterial type of pulsation. The electrocardiogram revealed P waves of large amplitude and a moderate degree of right axis deviation (Fig. 3).

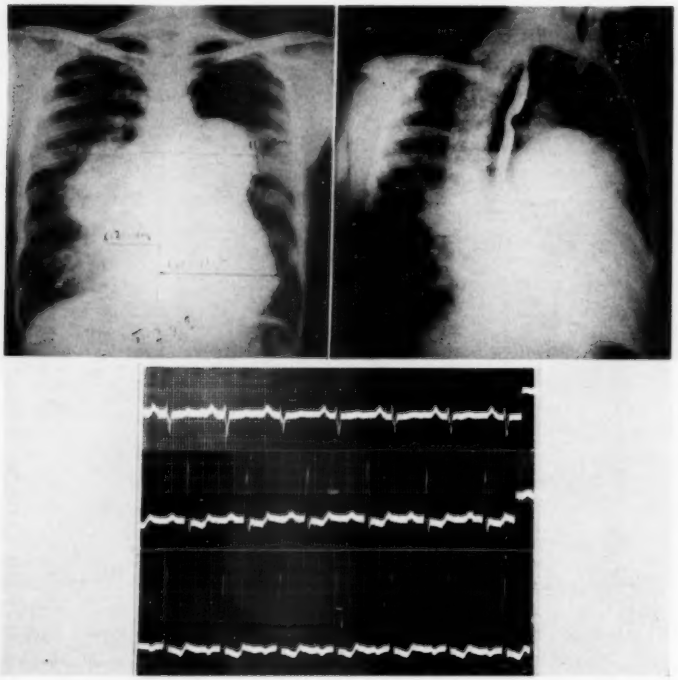

FIG. 3. X-RAYS OF CHEST FROM CASE 3 SHOWING ENORMOUS DILATATION OF THE PULMONARY ARTERY AND THE CONSPICUOUS HILAR SHADOWS CAUSED BY THE DILATATION OF THE RIGHT PULMONARY ARTERY

Course of the illness: In December 1935 the patient was admitted to the hospital because of myocardial insufficiency. After a short period of bed rest and digitalis she was discharged greatly improved.

In November 1936 she contracted a severe upper respiratory infection. Because of this illness she did not come to the dispensary to renew the digitalis. Five days after stopping digitalis she was brought to the hospital with extreme

dyspnea, orthopnea and coughing up of frothy sputum. The chest was filled with râles. The heart seemed totally irregular.[6] She was immediately given digitalis but with no avail. She died that day.

Clinical impression: Congenital heart disease with idiopathic dilatation of the pulmonary artery.

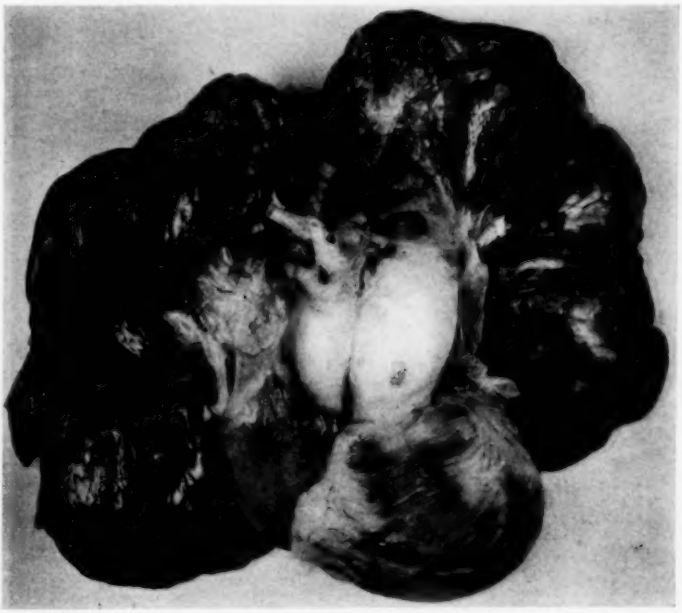

FIG. 4. SHOWS THE EXTERNAL APPEARANCE OF THE HEART AND LUNGS IN CASE 3. NOTE THE LARGE PULMONARY ARTERY. THE RIGHT VENTRICLE IS HYPERTROPHIED

Autopsy No. 15044, performed by Dr. Follis. The main interest centered about the heart. There was a definite precordial bulge. On opening the chest cavity the pericardial surfaces appeared to be smooth. The pulmonary artery was about twice the size of the aorta (Fig. 4). Their relations *in situ* were normal. The right auricle and ventricle were much enlarged. The great veins entered the right auricle in normal manner. There was a round, widely open foramen ovale, 5 cm. in diameter (Fig. 5); its margins were smooth and there was no valve. The

[6] Electrocardiogram not obtained.

tricuspid ring measured 15 cm. in circumference. The leaflets were thickened and the chordae tendineae shortened. The right ventricular wall was 1 cm. in width; the trabeculae were greatly exaggerated. The pulmonary leaflets were slightly thickened. The pulmonary ring measured 9.8 cm. in circumference. The pulmonary artery immediately above the ring was diffusely enlarged, measuring 14 cm. in circumference. Both branches were large, the one going to the right lung measured 14.1 cm. in circumference, while the left main branch was 12 cm. in circumference. The wall of the pulmonary artery and of its large branches was of normal thickness and contained only a few small sclerotic plaques. In com-

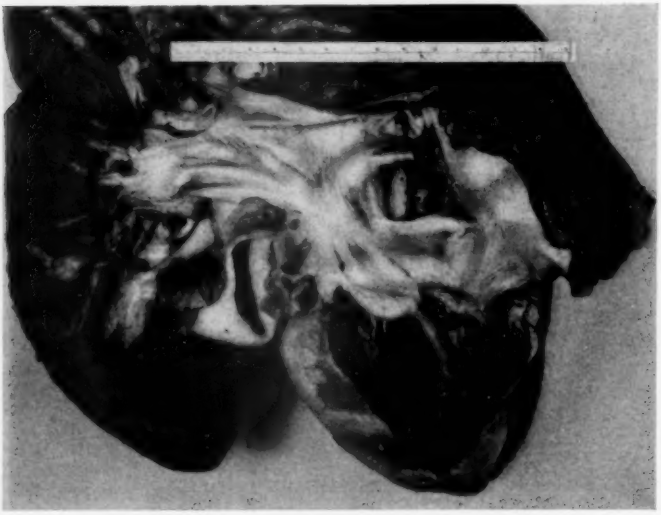

FIG. 5. SHOWS THE LEFT SIDE OF THE HEART WITH PATENT FORAMEN OVALE FROM THE HEART SHOWN IN FIGURE 4 (CASE 3)

parison with the right, the left auricle was small. There was no endocardial thickening. The mitral orifice just admitted the thumb. The mitral ring had a circumference of 5.5 cm. The leaflets were thickened and the chordae tendineae thickened and shortened. The left ventricular cavity was small; its wall was 1.4 cm. in thickness. The aortic leaflets were normal; the aortic ring measured 6 cm. in circumference. The aorta above measured 6.5 cm., while in the thoracic region it was 4.5 cm. in circumference and the abdominal aorta measured 3.5 cm. The ductus arteriosus was completely occluded. The interventricular septum was intact. The only abnormality in the other organs was chronic passive congestion.

Microscopically, the *myocardium* showed only moderate perivascular scarring. There was nothing to suggest that the rheumatic process had been recently active. The mitral valve was replaced by dense, hyaline connective tissue. The *pulmonary artery* had a normal amount of elastic tissue, as had the aorta; in neither were there any lesions. The *smaller pulmonary vessels* were normal. The *lungs* showed a fibrinous pleurisy and areas of pneumonia. The other organs showed typical changes of chronic passive congestion. There was no evidence of any arteriolosclerosis.

Anatomical diagnosis: Congenital malformation of the heart. Patent foramen ovale. Extreme dilatation of the pulmonary artery and its branches. Chronic rheumatic endocarditis of the tricuspid, aortic, and mitral valves. Mitral stenosis. Chronic passive congestion. Lobular pneumonia. Fibrinous pleurisy.

Case No. 4. G. I. (H. L. H. No. 95803) A white girl of 9½ years who was followed in the Harriet Lane Home from January 1936 till the time of her death in November 1936.

Past history: Birth was normal; no cyanosis was noted. The patient had scarlet fever at the age of 7 and two months later she had measles.

Present illness dates from March 1934 when, following measles, heart trouble was first discovered. During 1934 and 1935 she complained of occasional mild joint pains; she had four severe epistaxes and once developed an erythema circinatum. During these two years she had four attacks of pneumonia. In January 1936 she was admitted to the Harriet Lane Home because of decompensation.

Physical examination showed a very pale, frail, thin, markedly underweight, poorly developed girl of 9 years with a delicate and translucent skin. There was slight cyanosis of the lips but no clubbing or cyanosis of the extremities. There was widespread evidence of heart failure.

The *chest* showed a marked deformity with a pronounced precordial bulge. The *heart* was greatly enlarged. The point of maximum impulse was in the sixth interspace in the anterior axillary line. On palpation there was a very pronounced systolic thrill in the third left interspace close to the sternum and both a systolic and a diastolic thrill at the apex. On auscultation there was a very harsh systolic murmur in the third left interspace and a snapping pulmonic second sound. At the apex the first sound was loud and booming and there was both a systolic murmur and a prolonged, low-pitched mid-diastolic murmur. To the right of the sternum there was a pleuropericardial rub.

Clinical impression: Congenital malformation of the heart and probably a superimposed rheumatic infection.

Laboratory examination showed red blood count 5,600,000; white blood count 11,200 with 64 per cent polymorphonuclear cells. Hemoglobin 86 per cent. *Urine* showed only a faint trace of albumin. The *Wassermann* and *Mantoux* tests were both negative. The *sedimentation rate* of the red blood cells was normal and always remained normal. Both the *teleoroentgenogram* and the *fluoroscopic examination* showed great enlargement of the right side of the heart and a prom-

inent pulmonary conus; the aortic knob appeared to be small in both the A-P and oblique views. The entire esophagus was displaced backward but there was no displacement indicative of enlargement of the left auricle. The *electrocardiogram* was normal except for the conspicuous notching of S in Lead 1 and R in Leads 2 and 3; and a right axis deviation (Fig. 6). The *X-ray of the extremities* showed normal osseous development.

Course: The patient improved with bed rest and digitalis. About six weeks later she developed a bronchitis and a bronchopneumonia. After a stormy course the pulmonary infection subsided and she was transferred to St. Gabriel's Convalescent Home. Throughout the remainder of her life the child was a chronic invalid.

On September 25, 1936, she was readmitted to the hospital with the story that one week before she had had a rash and subsequently had developed a cough. On physical examination she again showed signs of bronchopneumonia. Four days later the patient developed a petechial rash over the thorax. Blood culture taken at this time showed a light growth of *streptococcus viridans*; three subsequent blood cultures were *sterile*. The pulmonary infection increased in severity and the patient progressively lost ground. She died of gradual cardiac failure. The day before death a friction rub was heard close to the sternum.

Final clinical impression: Congenital malformation of the heart—probably an interauricular septal defect and a questionable superimposed rheumatic infection.

Autopsy No. 15028, performed by Dr. Follis. The main interest centered about the heart. There was a definite bulge over the precordium. The pericardial sac contained 150 c.c. of straw-colored fluid and its surfaces were dulled by a fibrinous exudate, most marked on the right auricle. The heart weighed 265 gm. The right side of the heart was strikingly enlarged; the left auricle and ventricle were relatively small. The great veins entered the right auricle in normal fashion. The foramen ovale was a round opening, 3 cm. in diameter, with no semblance of a valve (Fig. 7). The right auricle was dilated and its wall was thickened. The tricuspid leaflets were delicate except for a few small, translucent vegetations on the posterior cusp. The chordae tendineae were normal. The tricuspid ring was dilated, measuring 11.5 cm. in circumference. The right ventricle was large, measuring 7 cm. in greatest dimension; its wall was 8 mm. in thickness. The pulmonary leaflets were delicate; the valve ring measured 6.4 cm. in circumference. The pulmonary artery above the valve was 6.5 cm. in circumference. Its larger branches in the lungs were conspicuous. The ductus arteriosus was completely closed. The interventricular septum was normal. The left auricle was small when compared with the right. In the position usual in rheumatic infections above the mitral valve, the endocardium of the left auricle was thickened. The mitral leaflets were thickened and their chordae tendineae were shortened and thickened forming a definite stenosis of the mitral orifice. The mitral orifice measured 5 cm. in circumference. The left ventricle was small; its wall measured 9 mm. in thickness. The aortic leaflets were delicate. The aortic ring measured 4 cm. in circumference; the aorta above was 3.5 cm. in cir-

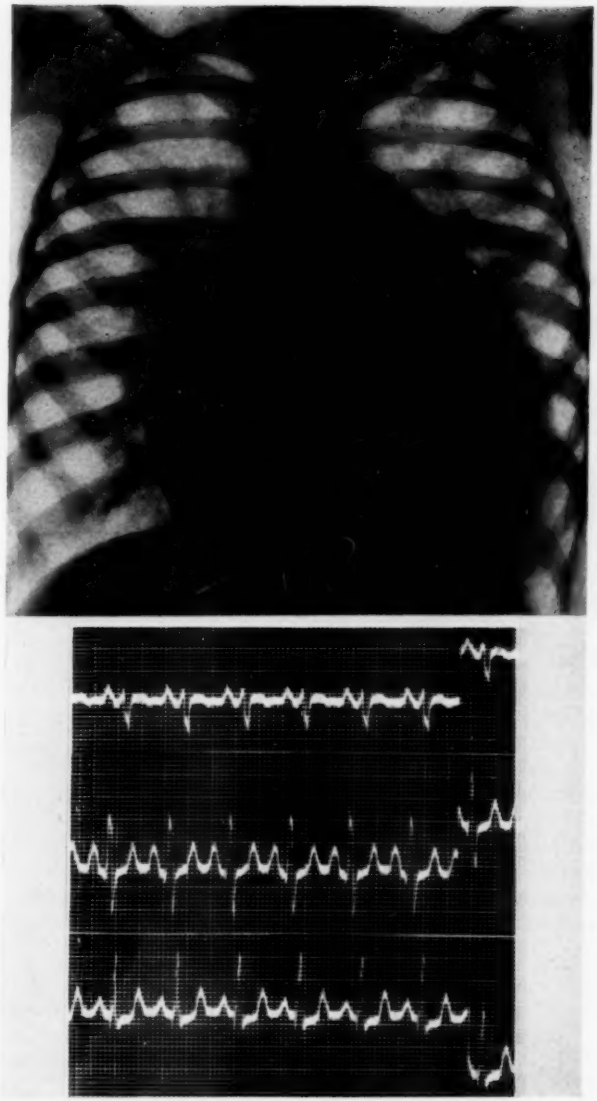

FIG. 6. ANTERIOR POSTERIOR VIEW OF CASE 4 SHOWING ENLARGEMENT OF RIGHT AURICLE AND RIGHT VENTRICLE, NARROW AORTIC SHADOW AND INCREASED PULMONARY SHADOWS

cumference. Just below the left subclavian artery there was a definite constriction; below this the thoracic portion of the aorta was only 2 cm. in circumference and the abdominal aorta measured 1.6 cm. The wall of the aorta was of normal thickness. The *liver* was somewhat enlarged, weighing 530 gm. The *spleen* was likewise enlarged; it weighed 90 gm. The *kidneys* were normal.

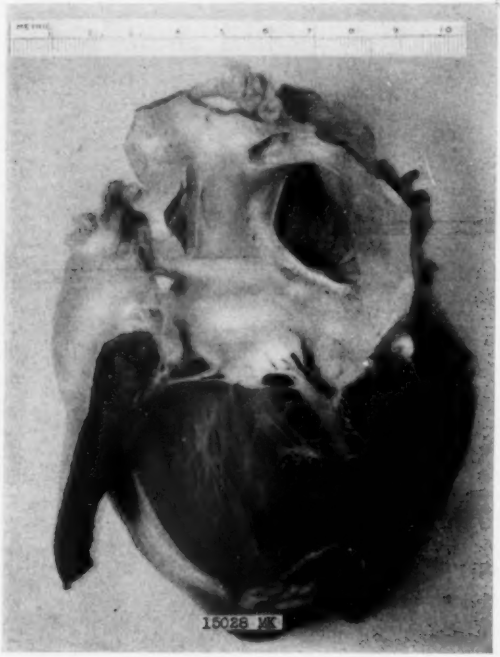

FIG. 7. SHOWS THE LARGE INTERAURICULAR SEPTAL DEFECT IN CASE 4. THERE IS DEFINITE STENOSIS AND THICKENING OF THE MITRAL LEAFLETS
(Photographs by Mr. Milton Kougl)

Microscopically, there were no fresh lesions indicative of an active rheumatic process. There was some increase in the connective tissue about blood vessels but no cellular infiltration. The mitral valve was made up of dense, hyaline fibrous connective tissue such as is characteristic of old rheumatic infections. The tricuspid valve showed some increase in connective tissue. The endocardium of the left auricle showed definite thickening but no cellular infiltration. The muscle fibers of the right and left ventricles were strikingly different, the former

being much larger than the latter. Sections of the *aorta* and *pulmonary artery* revealed a normal structure. There were heart failure cells in the pulmonary alveoli. The *smaller pulmonary vessels* showed only slight thickening of their walls. The *liver* showed a severe chronic passive congestion with increase in connective tissue about the central veins. Similar changes were seen in the *spleen*. There was no generalized arteriolosclerosis.

Anatomical diagnosis: Congenital malformation of the heart. Patent foramen ovale. Constriction of the aorta. Chronic rheumatic endocarditis of the mitral and tricuspid valves and the left auricle. Mitral stenosis. Fibrinous pericarditis. Chronic passive congestion. Lobular pneumonia.

DISCUSSION

The fundamental characteristic of this malformation is a gross defect in the foramen ovale permitting the free flow of blood between the two auricles. Although there is no absolute knowledge concerning the normal pressure in the two auricles, it is generally believed that the pressure is greater in the left auricle than in the right. Be that as it may, any condition which raises the pressure in the left auricle, such as stenosis or insufficiency of either the mitral or the aortic valve, or hypertension, forces the blood from the left auricle to the thin-walled, readily distensible right auricle. Thence the blood passes to the right ventricle, out by the pulmonary artery and back by the pulmonary veins to the left auricle; thereupon, the increased amount of blood returning to the left auricle raises the pressure in the left auricle and the blood is again shunted into the right auricle. The vicious cycle thus set up causes enlargement of the right auricle, right ventricle and pulmonary artery, whereas the left ventricle and aorta are spared. It is this left to right shunt through the interauricular septal defect which produces the series of pathological changes by which the condition may be recognized clinically.

THE PATHOLOGICAL CHANGES

The *pathological changes* are will illustrated in these four cases. In each instance the foramen ovale was widely patent; in none was there any suggestion of a valve or membrane covering the opening. In three of the cases there was stenosis of the mitral orifice; in the fourth there was mitral and aortic insufficiency. Thus in all four cases there was an obvious cause for the left to the right shunt.

The question immediately arises whether the valvular lesions had

been present at birth or whether they were the result of a superimposed rheumatic infection. In Case 2 all the evidence points toward an acquired condition. Although there was no stenosis of the mitral orifice, there was both clinical and pathological evidence of a mitral and aortic insufficiency and of a recent rheumatic infection. The other cases showed the structural changes in the mitral valve which are characteristic of an old rheumatic infection. Case 4, in addition, showed a thickened area in the left auricle in the location in which it so commonly occurs in acute rheumatic fever. These findings are strongly suggestive that the valvular lesion in each of the cases was an acquired condition resulting from a previous rheumatic infection.

On the other hand, all of the patients showed *chest deformity* which is strong evidence that the cardiac enlargement had occurred early in life while the bones were soft and pliable. This means that the initial enlargement was either caused by the patency of the foramen ovule *without* valvular lesion or that the rheumatic infection occurred at an extremely early age.

The characteristic enormous enlargement of the right auricle and the right ventricle was uniformly present. In each of the cases the wall of the right ventricle was increased in thickness.

Another characteristic feature of this malformation is the *relative enlargement of the pulmonary artery* (2, 39). Roesler (39) found that the pulmonary artery was always larger than the aorta; not infrequently the pulmonary artery was actually enlarged, and sometimes the enlargement was tremendous. This feature is well illustrated in our cases. In Cases 1 and 3 the circumference of the pulmonary artery, although within the limits of normality, was relatively enlarged, being 2 and 2.5 cm., respectively, greater than the aorta.[7] No. 2 was the only case in which the relative sizes of the pulmonary artery and the aorta were normal and in this case there was evidence of infection and unusual thickening in the wall of the pulmonary artery. In Case 4 the pulmonary artery was huge. The pulmonary ring measured 9.8 cm. and the artery immediately above the ring measured 14 cm. in circumference.

Slight enlargement of the pulmonary artery is readily explicable

[7] The pulmonary artery is normally about 1 cm. larger than the aorta.

on the basis of the hemodynamics of the circulation; the pulmonary artery carries a larger volume of blood than does the aorta. This explanation does not, however, seem sufficient to account for the tremendous dilatation of the pulmonary artery and its branches such as occurs in the true Lutembacher's syndrome (27).

In this syndrome (8, 17, 21, 24, 27, 35, 48, 49), which was exemplified in our third case, no obvious cause for the tremendous enlargement of the pulmonary artery has yet been found. In our case the lungs presented no abnormality. There was no sclerosis of the smaller pulmonary vessels as in a case reported by Mobitz (34), nor was there any infection or even any demonstrable abnormality[8] in the wall of the pulmonary vessel.

If the mitral stenosis were congenital in origin, it is conceivable that the left ventricle and aorta might remain small and the right ventricle and pulmonary artery become larger than normal. The correct explanation is, however, not as simple as this, because in Cases 1 and 3 in which the circumference of the aorta was less than normal the pulmonary artery was of normal size and in Case 4 in which the pulmonary artery was huge the size of the aorta was normal.

Although the cause for the enormous size of the pulmonary artery is obscure, it appears to be *ipso facto* a congenital malformation (9, 18, 35, 38, 44) which frequently accompanies a defect in the interauricular septum. The long-standing left to right shunt and the associated increase in the blood volume in the lesser circulation in all probability acts upon the underlying malformation to increase still further the tremendous enlargement of the pulmonary artery and its branches.

The last feature of this syndrome to be considered is the natural counterpart of the relative enlargement of the pulmonary artery, namely, *the aorta is small.* Not infrequently the aorta is not only relatively small but abnormally so. This was true in two of our cases (Nos. 1 and 4). Indeed, the aorta was so small as to raise the question of *hypoplasia.*

[8] Esser (15) reported a case of congenital enlargement of the pulmonary artery in which the wall of the vessel was deficient in elastic tissue.

In 1872, Virchow (46) described a narrow, thin-walled aorta rich in elastic tissue which was associated with chlorosis. This type of aorta was called "hypoplastic aorta" or "aorta angusta"; it was considered in itself to be a cause of cardiac failure. In 1907, von Ritook (47), in an extensive discussion of the subject, expressed the opinion that hypoplasia of the aorta was not a disease entity but represented a poor development of the arterial tree which rendered the individual unable to withstand severe illness or excessive strain. During the World War Kaufmann (25) made an extensive study of the variation in the width of the aorta of normal individuals. She found a wide variation in the size of the aorta at all ages and expressed skepticism concerning the existence of a "hypoplastic" aorta. Mainly on the basis of Kaufmann's work, Jorès (23) concluded that the "aorta angusta" should not be considered as a disease entity.

In two of our cases, to repeat, the aorta was definitely smaller than normal. In each instance the wall appeared to be of normal thickness and of normal structure; hence they do not fulfill the criteria of "hypoplasia." They are, however, in direct accordance with von Ritook's conception of a poor development of the arterial tree. As will be shown presently, further confirmation of this conception is to be found in the physical development of these individuals.

CLINICAL FINDINGS

The *clinical findings* are in direct accord with the pathological changes. The two fundamental features, *i.e.*, the great enlargement of the right side of the heart and the relatively small aorta are reflected in the body build. Indeed, the appearance of the patient may be so characteristic as to suggest the diagnosis at sight. Left-sided chest deformity is the rule. This is produced by the marked enlargement of the right ventricle which causes the heart to press against the anterior chest wall.

If the left to right shunt is established in early life, the physical development shows, in addition to the chest deformity, certain distinctive features. The patient has the frail slender build of the "gracile habitus." The skin has a semitranslucent appearance and is of a fine delicate texture. The bony structure is lighter than that of the average individual and the onset of puberty is delayed. The

explanation of these features is found in the left to right shunt, by which the pulmonary circulation receives more blood than normal and the systemic circulation actually receives *less* than its normal quota of blood. Thus the body which depends for its nourishment on the systemic circulation is starved.

Inasmuch as the shunt is an arterio-venous one the malformation belongs in the group of the *cyanose tardive*. Clubbing and cyanosis are absent. Not infrequently there is slight acrocyanosis caused by the increased deoxygenation in the peripheral tissues associated with the diminished blood volume and increased utilization of oxygen in the systemic circulation. There is, however, never the intense cyanosis and concomitant clubbing of the venous-arterial shunt. As in all malformations belonging to this group, the possibility exists of a transitory or terminal shunt reversal, such as occurred in Case 2 during the final illness. Such a shunt reversal will cause intense cyanosis, but it is never of sufficient duration to produce clubbing. Indeed, if clubbing is present, it is indicative of some additional complicating factor which has caused a reversal of the shunt.

On *physical examination* the heart is found to be greatly enlarged; the apex beat is displaced downward and outward; it frequently lies in the sixth interspace outside the anterior axillary line. Because of the great enlargement of the right auricle the cardiac dulness to the right of the sternum is also greatly increased.

The *murmurs* and *thrills* in these cases behave in a manner similar to all murmurs of congenital malformations of the heart in which there is a communication between the two circulations; such signs always depend upon the relative pressure in the greater and the lesser circulation. In the well compensated case in which the pressure in the left auricle is higher than in the right there is a pronounced murmur and thrill to the left of the sternum in the *second* and *third* interspaces. The murmur is *systolic* in time and closely resembles that of an interventricular septal defect except that its point of maximum intensity is located slightly higher up on the chest than is usual with the *maladie de Roger*.

The intensity of the murmur is not as constant as that of an interventricular septal defect. Indeed, there may be great variation in the intensity of the murmur not only when one case is compared with

another but when the same individual is observed under different circumstances. For example, in Case 1 no murmur was noted until the terminal illness. The reverse may occur. With increasing pressure in the lesser circulation, the difference in pressure between the two circulations may decrease; this in turn would be accompanied by a decrease in the intensity of the murmur and thrill. Furthermore, with the development of congestive heart failure and the great increase in the pressure in the lesser circulation, the murmur may entirely disappear. Two such cases have been reported by Cassio and Arana (6); these cases were, however, complicated by auricular fibrillation. The absence of murmurs in these cases may have been due to the absence of the normal auricular contractions.

The *increased pressure in the lesser circulation* causes an accentuation of the *pulmonic second sound*. In older persons the right ventricle and the pulmonary artery not infrequently become so greatly dilated that a *relative pulmonary insufficiency* develops, causing an early diastolic murmur along the left sternal border such as occurred in Case 3. It is also interesting to note that in Case 3 there was a thrombus attached to the pulmonary artery. A similar finding occurred in a case in Boston in which an enormously dilated pulmonary artery was virtually occluded by a thrombus.[9] It should be borne in mind that enormous dilatation of the pulmonary artery may lead to venous stasis and thrombus formation (2, 30, 44, 48).

In cases with *mitral stenosis*, either congenital or acquired, both a systolic and a dull mid-diastolic murmur are usually heard at the apex. Nevertheless, it is unusual for the mitral stenosis to cause enlargement of the left auricle because the defect in the interauricular septum permits an easy egress of blood from the left auricle to the right. Indeed, in this malformation, it is the *right* auricle which bears the strain caused by the stenosed mitral valve. Other valvular lesions, such as mitral insufficiency or aortic insufficiency, produce their characteristic murmurs and thrills.

The *roentgenogram is often of diagnostic aid*. The distinctive features in the x-ray pictures (5, 11) are (1) the great enlargement of the right auricle and right ventricle; (2) the prominent pulmonary conus; (3) the small aortic knob; (4) the increased hilar shadows. It is to

[9] Kindness of Dr. T. Mallory at Boston City Hospital.

be noted that according to whether or not the pulmonary artery is greatly dilated, the x-ray shadows are entirely different. Case 4 exemplifies the type which McGinn and White (30) considered to be characteristic. The x-ray picture in Case 4 is almost the exact duplicate of theirs and also of those of Wahl and Gard (48) and of Roesler (39). Each shows the four distinctive features.

An illustration of the other type of X-ray is that shown in Case 3.[10] This X-ray is closely similar to that originally described by Assman (5) and is the characteristic picture seen in the true Lutembacher syndrome. The most conspicuous feature in these cases is the dense shadow cast by the pulmonary artery and its branches. This shadow may be so large as to be mistaken for a mediastinal tumor (12, 30, 48).[11]

Fluoroscopic examination is of great help in determining the nature of the wide mediastinal shadow. Unless the condition has progressed to thrombus formation these shadows will pulsate; this is a condition which causes a true "dancing hilum." The conspicuous pulsation in the region of the pulmonary conus frequently necessitates differentiation between this malformation and a patent ductus arteriosus. The absence of the characteristic machinery murmur and the disproportionate enlargement of the right side of the heart combined with the pulsation in the lung fields aids in making the correct diagnosis.

The two oblique views substantiate the impression that the enlargement is mainly of the right side of the heart. On rotating the patient into the left anterior oblique the right ventricle is usually seen to project against the anterior chest wall. The enlargement is often so great as to displace the left ventricle backward and thus give it the appearance of sharing in the enlargement. In the right anterior oblique the entire esophagus may be pushed backward. It is unusual, however, to see specific enlargement of the left auricle even in the presence of a mitral stenosis because, as previously mentioned, the *right* not the left auricle bears the brunt of the strain caused by the mitral stenosis.

[10] The X-ray picture is almost identical with those shown by Assman and Oppenheimer.

[11] Also in Dr. Mallory's case.

The *electrocardiogram* shows high P waves and usually there is a definite though not pronounced right axis deviation. The right ventricular preponderance is not as great as might at first blush be expected because the tremendous enlargement of the right ventricle is not purely hypertrophy but results from a combination of dilatation and hypertrophy. It is not unusual to have evidence of abnormal intraventricular conduction such as occurred in Cases 1 and 2.

There are, in addition to these physical findings, certain sequelae of such common occurrence as to be of diagnostic significance.

Cardiac arrhythmias of all forms are common. Not only are extrasystoles frequent but also paroxysmal tachycardia, auricular flutter (4), auricular fibrillation (6, 10, 39), ventricular escape and even complete dissociation. The frequency with which these arrhythmias occur is well illustrated in our cases. Case 1, in addition to numerous extrasystoles, was thought to suffer from paroxysmal tachycardia. Case 2 was known to have an intermittent complete dissociation and frequent periods of ventricular escape. Case 4 at the time of the last admission was suspected of a transitory auricular fibrillation. Although not appreciated at the time, paroxysmal tachycardia and transitory auricular fibrillation, when they do occur, are of diagnostic aid because this is the only congenital malformation in which these arrhythmias are common.

A superimposed rheumatic infection is the most frequent complication. This occurs in 60 to 75 per cent of all cases (39). The rheumatic manifestations may be so conspicuous as to mask the underlying congenital malformation, easily leading to a mistaken diagnosis of uncomplicated rheumatic heart disease. This mistake was made in our first case. The opposite mistake was made in the second case; the original diagnosis was that of a congenital malformation of the heart and it was not until the boy's terminal illness that the existence of a rheumatic infection was recognized.

Case 4, although obviously suffering from a congenital malformation of the heart, gave a history suggestive of acute rheumatic fever. The frequent association of these two conditions indicates that in cases where the differential diagnosis lies between a congenital malformation of the heart and acquired heart disease, one should bear

in mind the possibility of a rheumatic infection superimposed upon this particular malformation.

Although *subacute bacterial endocarditis* is notoriously common in cases of congenitally malformed hearts and on valves previously damaged by a rheumatic infection, *no case of subacute bacterial endocarditis has as yet been reported in association with this malformation.* It is of great interest that Case 4 had a transitory streptococcus viridans bacteremia or septicemia, but at autopsy there were no signs of a subacute bacterial endocarditis.

Pneumonia and pulmonary infections and pulmonary emboli are frequently encountered. Again our cases are not exceptions. In Case 1 the patient had two attacks of so-called "dry pleurisy;" in Case 3 the woman died of cardiac failure precipitated by a respiratory infection; in Case 4 the patient suffered from repeated attacks of pneumonia and died of a combination of cardiac failure and pulmonary infection.

In addition to the features thus exemplified by our cases, there are a few others which deserve mention. The first is the curious preponderance of females over males, the ratio being 2 to 1 (3, 39). The second is the relative longevity of the majority of the patients (16). Third, it is not unusual to obtain the history that the patient was not known to have any cardiac abnormality; after years of apparent health some minor illness precipitated the sudden onset of symptoms which was followed by progressive cardiac failure. Finally, because of the great dilatation and increased pressure in the right auricle, this malformation is one of the conditions in which there exists the danger of paradoxical emboli (3, 14, 19).

SUMMARY

(1) The essential pathological findings are a widely patent foramen ovale, great dilatation and hypertrophy of the right auricle and the right ventricle and relative enlargement of the pulmonary artery and its branches and a small left ventricle and aorta. This syndrome is usually associated with some condition, such as a left side valvular lesion or hypertension, which causes increased pressure in the left auricle. The characteristic enlargement of the right auricle and

ventricle may, however, occur in the absence of any demonstrable increased strain on the left side of the heart.[12]

[12] Since this report was written, another case with an interauricular septal defect has been examined at autopsy which illustrates that left-sided cardiac strain is not an essential feature in this syndrome.

C. H. (H. L. H. No. 33834) A negro girl who was first seen in The Harriet Lane Home at 5 weeks of age and considered to be a normal infant. When next seen at 3 years of age, the examination of the heart revealed a loud basal systolic murmur and thrill. The child was recognized as having a congenital malformation of the heart. A note was made that she probably had a patent ductus arteriosus without a diastolic murmur because the murmur was "of maximum intensity in the first and second interspaces and much too high for an interventricular septal defect." At the age of 7 years, extrasystoles were first noted and were present thereafter on numerous occasions. The teleoroentgenogram showed the heart to be slightly enlarged; its contour was not remarkable except in one plate in which there was enlargement of the right auricle. On fluoroscopic examination, in the region of the pulmonary conus and great vessels, there were conspicuous pulsations which were thought to be consistent with a patent ductus arteriosus. These findings are, however, equally characteristic of an interauricular septal defect. The electrocardiogram was normal. The blood pressure at 14 years of age was 102/60. In brief, the clinical findings were similar to those of Case 2. The differential diagnosis lay between a patent ductus arteriosus and an interauricular septal defect.

The patient never had any complaints referable to her heart, nor did she suffer from any manifestations of acute rheumatic fever. In 1938, at 15 years of age, she was admitted to the gynecological service with an acute peritonitis secondary to an acute salpingitis. On admission she also had a severe respiratory infection and some congestion in her lungs. Operation was imperative and was performed under cyclopropane anaesthesia. The patient developed lobar pneumonia and on the second day after operation died.

At autopsy (No. 15728) the heart was found to be enlarged with distinct hypertrophy of the right auricle and ventricle. There was an interauricular septal defect 1 cm. in diameter with no suggestion of a valve or membrane. The pulmonary valve leaflets were thickened but the valve was competent. The other valves were normal. Microscopically, there was no evidence of rheumatic infection.

In summary: The patient showed clinical signs of a congenital malformation of the heart which was first recognized at the age of 3 years by the presence of a harsh basal systolic murmur and thrill. There were no complaints referable to the heart. The patient died from a postoperative pneumonia. The autopsy revealed a gross defect in the interauricular septum and although there was no demonstrable strain on the left side of the heart, there was dilatation with hypertrophy of the right auricle and right ventricle.

(2) On physical examination the significant findings are as follows: A frail individual with left-sided chest deformity, marked cardiac enlargement of the right auricle and the right ventricle, and usually a harsh systolic murmur and thrill in the second and third left interspaces. Clubbing and cyanosis are absent.

(3) The x-ray picture shows four distinctive features, *i.e.*, great enlargement of the right side of the heart, a prominent pulmonary conus, a small aortic knob and increased hilar shadows.

(4) The sequelae which are of such common occurrence as to be of diagnostic aid are cardiac arrhythmias, superimposed rheumatic infections, pulmonary infections, paradoxical emboli and the freedom from subacute bacterial endocarditis.

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THE METABOLISM OF THE ISOLATED LIVER[1]

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As the subject of the third and last lecture which I shall have the honour of delivering to you on this occasion, I have chosen the metabolism of the isolated liver, as studied on isolated, artificially perfused livers. Together with various collaborators I have occupied myself with investigations in this field for the past three years. In choosing this topic for my last lecture, I have done so partly because some of the problems into which these investigations have led us are very closely associated with those on which I dwelt yesterday. For example, the experiments on alcohol oxidation to which I then referred were a part of these investigations.

Having regard to the time I have at my disposal I shall not go deeply into the technique employed for the artificial perfusion of isolated livers in my institute. I shall merely point out that this technique may safely be regarded as adequate for the following reasons. After a rather heavy initial fall of oxygen consumption in the first ten minutes after the liver has been isolated, the oxygen consumption remains fairly constant for a long time. The average oxygen consumption in a cat liver of 80 gm. is 2.5 cc. per minute. As a general rule our experiments begin twenty minutes after isolation of the liver, and the experimental period proper does not extend beyond two hours. The lactic acid content of the perfusion blood remains low throughout the entire period—indeed extraordinarily low in experiments with cat livers (3 to 5 mg. %). This may be taken as a sign of adequate oxygen supply. Under suitable conditions it is always

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possible to obtain a considerable storage of glycogen. In one respect only does the isolated liver appear to behave abnormally, for there is a steady, slow diffusion of phosphate from the liver to the perfusion blood, in which the inorganic phosphate consequently rises evenly throughout the entire period.

Our experiments were started as a kind of continuation of some that were made five or six years ago, at The Institute of Medical Physiology, by Dr. Niels A. Nielsen (4), who also at first took an active part in this work. The most important outcome of Nielsen's researches was the demonstration that, in perfusion experiments, it is not possible to augment the already active storage of glycogen in isolated rabbit and dog livers by adding insulin to the blood.

In experiments on the administration of insulin to isolated livers, it has been found necessary to employ preparations of crystalline insulin, because the ordinary commercial preparations have a very marked glycogenolytic effect. We are much indebted to Professor Abel for the help he has given us by supplying us with very liberal amounts of the appropriate kind. From Professor Best's laboratory, too, we have procured crystalline insulin made by Dr. Scott.

I might mention very briefly that in our experiments we have observed a distinct difference between the two preparations, though as to potency they were equal. The difference was, that whereas the Abel insulin has no glycogenolytic effect at all, that effect is quite pronounced in the Scott insulin.

The result I mentioned of Dr. Nielsen's experiments with rabbit livers was confirmed by us in numerous experiments. In these we added glucose continuously to the blood, at such a rate that, as far as possible, the blood sugar concentration was kept constant. Under such conditions it is easier to observe even small variations in the rate at which glucose disappears from the blood than if the blood sugar is constantly falling. Another effect obtained by this means is to eliminate the influence which changes in the blood sugar concentration may have on the rate of deposition.

As the explanation of the fact that one cannot obtain any effect of insulin on the isolated rabbit liver—a fact which I shall regard as having been sufficiently established—one might imagine that in such an isolated liver the deposition of glycogen proceeds at a maximum

velocity beyond which it cannot be further increased. This suggestion is so much the more natural, because the liver in the intact organism undoubtedly is constantly under the influence of hormones, so that we may speak of a certain balance between influences which promote the mobilisation of glycogen and the deposition of that substance, respectively. For the purpose of learning something more about this question, we have undertaken experiments in which an attempt was made to check the glycogen deposition by continuous administration of small quantities of adrenalin, in order to investigate whether it was possible to observe any insulin effect under such conditions. Though these experiments, like each of our attempts to demonstrate an effect of insulin on the isolated liver, turned out negatively, I shall show you some curves from them (fig. 1). You see that, after an initial fall in the blood sugar, the administration of adrenalin begins at the arrow marked A. From that point and till the end of the experiment, adrenalin is administered continuously in quantities of 1 gamma per minute. We chose this adrenalin dose in order that the effect would be distinct but moderate, thus giving the insulin a fair chance to assert itself. It will be seen that the immediate effect of the adrenalin is, as would be expected, an increase of the blood sugar. The increase, however, soon ceases. The curve forms a plateau which is maintained for some time, whereupon the blood sugar, despite the continued supply of adrenalin, falls at a rate which conforms closely to the rate prior to the administration of the adrenalin. Such curves are obtained with absolute regularity. I am unable to explain why the adrenalin effect gradually wears off. At the conclusion of the experiment the livers contain ample glycogen. The carbon dioxide tension in the blood is kept constant and a change in the pH of the blood may be considered out of the question. We have now tried giving insulin a short time before or a short time after the commencement of the adrenalin administration, but in no case has the course of the blood sugar curve been affected by it.

At a fairly early stage of our experimental work in this field we began to employ cat livers for our perfusion experiments. We then found that there is a very pronounced and curious difference between cat and rabbit livers with regard to their ability to store glycogen (2). In experiments with isolated rabbit livers the blood sugar falls rapidly

and the glycogen content rises rapidly. The lactic acid concentration in the blood remains fairly constant at values about 15 to 20 mg. %. In similar experiments with isolated cat livers the blood sugar remains constant, or rises slightly. No glycogen is stored, and the blood lactic acid concentration remains extraordinarily low throughout the experiment.

In reality, the values found for the blood lactate are so low that, in view of the fact that our methods of lactic acid determination are

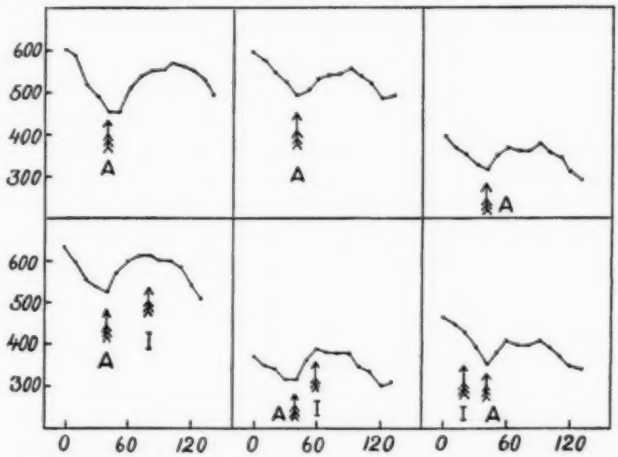

FIG. 1. Blood sugar concentrations in blood perfused through isolated rabbit livers. At "A" continuous addition of adrenalin (1 gamma per minute) is started. At "I" 10 international units of crystalline insulin (Abel) are added to the blood.

not absolutely specific, it is doubtful whether there is lactic acid in the blood at all in these experiments.

I must point out that the fall of glucose in the perfusion blood in experiments with rabbit livers is so considerable, that the glucose oxidation in the liver cannot be of any essential significance to it. There can scarcely be any intense oxidation of carbohydrates in a rabbit liver. In experiments with rabbit livers there is on the average a respiratory quotient of 0.80. In experiments with normal cat livers we get a quotient of 0.55 to 0.60. Thus, although there is a

certain difference in the respiratory quotient of the two animals, the marked difference in the utilization of glucose must be the result of a very different ability to store glycogen.

As the isolated cat liver spontaneously behaves towards glucose as one would naturally expect a diabetic liver to do, we have made a number of experiments for the purpose of inducing a deposition of glucose in the form of glycogen by adding insulin to the blood. In these experiments we have varied the insulin doses within wide limits. We have tried injecting the insulin into the animal before isolating

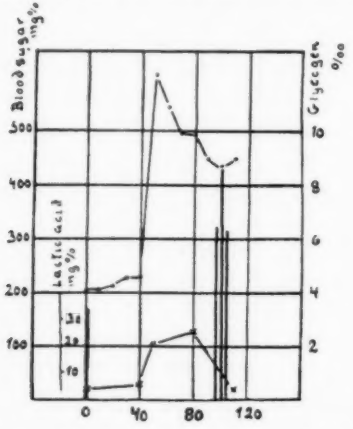

FIG. 2. Blood sugar and lactic acid concentration in blood perfused through an isolated cat liver. At 40 minutes 1 gram of fructose is added to the blood. The vertical columns indicate the glycogen content in the liver tissue.

the liver, and we have tried the continuous administration of insulin in alternate doses throughout the entire period. In no case did we succeed in inducing a deposition of glucose as glycogen in an isolated cat liver. That this does not mean that glycogen cannot be stored at all in the isolated cat liver, is shown by the fact that the administration of fructose or of lactic acid causes a very marked deposition of glycogen—in the cat liver too. Figure 2 shows the curves from an experiment in which fructose (1 gm.) was administered. After the initial sudden rise, the blood sugar decreases rapidly, and glycogen is stored in the liver. Immediately after the addition of the fructose the

lactic acid content in the blood rises, and it remains relatively high until the fall of the blood sugar curve has come to an end. A comparison between the reducing power and rotation power of the blood shows that, at this point, the entire amount of fructose still remaining in the blood has been converted into glucose.

We see, then, that the deposition of glycogen continues only as long as there is fructose in the blood, and as long as the increased lactic acid in the blood indicates that lactic acid is being formed. I attach a good deal of importance to this latter point, for I am inclined to the belief that lactic acid formation is an intermediate phase in the conversion of the hexoses to glycogen. At this juncture, however, I shall not venture on a discussion of this interesting problem.

The glycogen deposition demonstrable in an isolated cat liver, after fructose or lactic acid has been added to the blood, is not increased by the addition of insulin. Thus no insulin effect on the isolated liver can be made manifest in these experiments either. Now the question may be asked whether the cause of the absence of any insulin effect on the liver in our experiments might not be, that the insulin preparations employed are quickly destroyed under the experimental conditions. That possibility may be ruled out, however, for with great regularity we obtain a distinct insulin effect in experiments with hind-limb preparations, in which exactly the same technique is employed. Thus if insulin is destroyed in our liver experiments it cannot be due to the experimental conditions, but must be due to insulin being rapidly destroyed in the liver itself. In that case insulin can not exert any effect on the liver even under normal conditions.

I confess that I have been greatly surprised that under the extremely varied experimental conditions employed, we have never been able to demonstrate any insulin effect on the liver. Nevertheless, I consider that the necessary conclusion to be drawn from these experiments is that insulin does not exercise any direct effect on the liver. I shall in a few words describe an observation we have made which to some extent bears out that conclusion.

In our hind-limb experiments insulin, besides speeding up the transfer of glucose from blood to muscles, also causes a fall in the concentration of inorganic phosphates in the blood. The demonstration of this well-known insulin effect in isolated hind-limb preparations

shows, incidentally, that what is involved is a direct insulin effect, and is not, as was once supposed by Cori and Cori (1), an effect of a compensatory increased adrenalin production under insulin hypoglycaemia. This effect, which doubtless is closely connected with insulin's main effect, the effect on the metabolism of carbohydrates, has never been observed in experiments with livers. I consider this of some importance, because in the liver too the carbohydrate me-

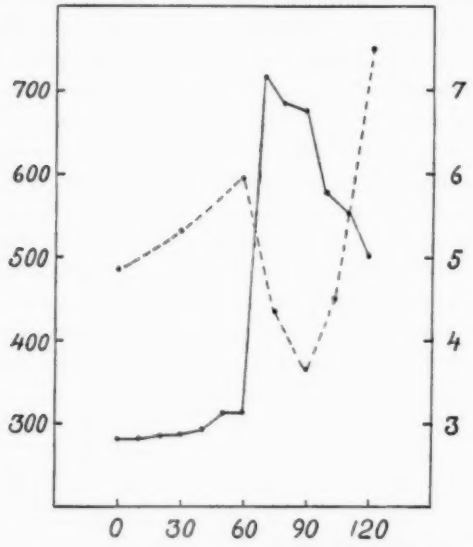

FIG. 3. Blood sugar (·———·) and inorganic phosphorus (·----------·) in blood perfused through isolated cat liver. At 60 minutes 1 gram of fructose is added to the blood. Abscissa: time in minutes. Left ordinate: blood sugar in mg. %. Right ordinate: inorganic phosphate in mg. % P.

tabolism affects the inorganic phosphate content of the blood. This appears from the fact that, as I said, in experiments with cat livers perfused with blood to which only glucose is added, we see a pronounced increase of the inorganic phosphates of the blood throughout the entire experiment, whereas the addition of fructose, which is utilized and stored in the liver, leads to an immediate heavy fall in the inorganic phosphate. I take the liberty of showing you a curve from an experiment to illustrate that fact (fig. 3).

By means of experiments with isolated livers from pancreatectomized cats, we have also attempted to throw some light on the question of the effect, or lack of effect, of insulin on the carbohydrate metabolism of the liver. The experiments were made 48 hours after the pancreatectomy, and the animals were not fed in the time between the operation and the experiment. They were in quite good condition, but showed very marked glycosuria. Since the normal cat liver does not store glucose as glycogen, the experiments with "diabetic" livers were performed with the addition of fructose. The first series of this kind comprised only five experiments. In two of them the blood used was from pancreatectomized cats, in three it was from normal animals. All five experiments agreed in showing that, after the addition of fructose, the storage of glycogen proceeds exactly as in preparations from normal animals. In other words, it was not possible to find any difference between livers from normal cats and those from pancreatectomized cats. This result too may be said to argue in favour of the view that insulin does not exert any direct effect on the carbohydrate metabolism of the liver.

Subsequent experiments have shown, however, that sometimes no storage of glycogen can be induced after adding fructose in experiments with livers from pancreatectomized cats. The fact is that there can be no storage if the liver contains much fat in addition to being poor in glycogen. If fat cats are pancreatectomized, the liver will reveal a high degree of fat infiltration even after only 48 hours. The metabolism of such a liver presents many features of interest on which I would like to dwell a few moments.

As I have said, the normal isolated cat liver has a respiratory quotient of 0.55–0.60. A fat-infiltrated liver from a pancreatectomized cat has a respiratory quotient generally about 0.30. The respiratory quotient of even the normal cat liver is so low as to indicate that partial oxidations must take place in it, and this is still more evident as regards the fat-infiltrated liver.

In experiments with isolated cat livers one always observes some fall in the blood alkali reserve while the experiment is proceeding. This fall is the more pronounced the higher the fat content of the liver. In experiments with livers with great fat infiltration, the alkali reserve may fall, for instance, from 29 cc. to 12 cc. The rather low initial value is due to our employment of blood slightly diluted

with saline. Usually the hæmatocrit number is about 25. There is an unmistakable parallelism between the value of the respiratory quotient and the fall in the alkali reserve: the lower the quotient, the greater the fall in the alkali reserve. This is due to the fact that the main cause of the very low respiratory quotients, as shown by Dr. Blixenkrone-Møller,[2] must be seen in a formation of ketone bodies, especially β-hydroxybutyric acid.

According to the classical view, β-hydroxybutyric acid is formed by the so-called β-oxidation, by which one molecule of β-hydroxybutyric acid is formed from every molecule of fatty acid, whereas the rest of the molecule is oxidized to carbon dioxide and water, according to the equation:

$$C_{15}H_{31}COOH + 18\tfrac{1}{2}O_2 = CH_3CHOH\,CH_2\,COOH + 12\,CO_2 + 12\,H_2O$$

According to this equation, the formation of 100 mg. of ketone bodies requires 398 cc. oxygen and gives 258 cc. carbon dioxide, so that the respiratory quotient should be 0.65. However, the much lower respiratory quotients found show that ketone formation cannot proceed according to this equation. In an arbitrarily chosen experiment on a liver from a pancreatectomized cat, there was a total ketone formation of 425 mg. in a period of 2 hours, and a simultaneous oxygen absorption of 365 cc. In that experiment the respiratory quotient was 0.32. Had the ketone formation proceeded according to the equation, the formation of 425 mg. β-hydroxybutyric acid would have required an oxygen quantity of 1693 cc., whereas actually it amounted to only 365 cc.

Therefore there can be no doubt that a much greater fraction of the molecule to the fatty acids, and presumably the whole molecule, is converted into β-hydroxybutyric acid. A conversion of the whole molecule to β-hydroxybutyric acid would proceed according to the equation:

$$C_{15}H_{31}COOH + 5\,O_2 = 4\,CH_3CHOH\,CH_2\,COOH$$

according to which only 27 cc. oxygen are required to form 100 mg. of β-hydroxybutyric acid. By this process no carbon dioxide is formed, and therefore the respiratory quotient can be very low, as it

[2] Now published: *Zeitschr. f. physiol. Chem.*, **252**, 112, 1938.

will depend on the extent of the ketone formation in proportion to the other metabolic processes.

In addition to ketone formation, two other processes may help towards making the respiratory quotient low, namely, a desaturation of fatty acids and a carbohydrate formation from fat. The former of these two processes is only small in extent, as may be seen in determinations of the iodine number of the liver fat in samples taken at the beginning and at the conclusion of the experiment. On the other hand, in all experiments with glycogen-poor livers there is quite a considerable new-formation of carbohydrate, evidenced by a rise in the blood sugar concentration. The glucose does not come from the liver glycogen, since the glycogen content in the liver in these experiments is, as I have said, very small and as a matter of fact remains constant throughout the experiment. In some cases, indeed, one observes a slight increase of the glycogen content. Nor can the glucose come from lactic acid, because the lactic acid content in the blood is always very low in experiments with isolated cat livers, and, what is more, remains very constant. In many cases the glucose formation is no greater than may be accounted for by the glucose which may come from metabolized protein. The protein metabolism of the isolated cat liver, measured by the rise in the blood urea concentration, is relatively high. A liver of 60 to 70 grams can convert 200 to 250 mg. protein in the course of two hours. Compared with a liver as large as a human liver, this corresponds to about 50 g. protein in the course of 24 hours. As no amino acids are added to the blood in our experiment I am inclined to consider this a fairly high protein metabolism. In some experiments, however, the D:N quotient is so high that some of the glucose may have been formed from fat. However, the highest value observed for the D:N quotient is only 10.6, which, in view of the uncertainty attaching to determinations of this quotient in experiments of this kind, is not convincing proof of a formation of carbohydrate from fat. Consequently, the carbohydrate formation which definitely takes place in glycogen-poor, isolated cat livers can scarcely be of much significance as a cause of the low respiratory quotients. As I have said, the main cause of these low quotients is the intense formation of ketone bodies.

As we have seen, the fat-infiltrated liver of a pancreatectomized cat

is remarkable for its pronounced ketone formation and a carbohydrate formation of moderate extent. These properties, however, can scarcely be classified as diabetic in the sense of "due to insulin deficiency." The addition of insulin to the blood in experiments with fat livers from diabetic animals causes neither a reduction of the glucose formation nor a reduction of the ketone formation and a rise in the respiratory quotient. One has to admit that the explanation of this might be, that the fat metabolism cannot change quickly enough to allow the change to be observed within the rather limited experimental period which we are obliged to employ in our work. Therefore it may be of greater importance that these properties cannot be said to be characteristic of the liver of a diabetic cat, but that in reality they are merely characteristic of a liver which simultaneously contains much fat and is poor in glycogen.

Formation of ketone bodies is also observable in the normal cat liver. If the liver contains merely moderate quantities of glycogen, the ketone formation is moderate. Under such circumstances the new-formation of carbohydrates is difficult to appraise, as a moderate rise in the blood sugar may be due to the breakdown of liver glycogen, which can be quantitatively determined only with limited accuracy. If while it contains a moderate quantity of glycogen the liver contains much fat (9 per cent or more), the ketone formation is intense. The same is the case with livers with a moderate fat content but at the same time containing very little glycogen, that is to say, livers with a glycogen content of less than 1 per thousand. And finally, ketone formation is most intense in livers which at the same time contain much fat and are almost free from glycogen. There is no difference with regard to carbohydrate formation in the last-named categories of livers.

In other words, then, there is an even change from the metabolism observable in the liver of a fully normal cat to the metabolism in the liver of a fat cat 48 hours after a pancreatectomy.

In experiments with a glycogen-poor, fat liver from a normal cat, it is also to be seen that the addition of fructose is incapable of effecting a storage of glycogen, or of producing a rise in the respiratory quotient, such as is seen in experiments with livers containing little fat, whether they are from normal or from pancreatectomized

animals. Nor can we regard the failure to store glycogen as being characteristic of a "diabetic" liver.

If what I have said be compared with the fact that no one has ever succeeded in showing that insulin has a direct effect on the liver, the following assumption would seem to be justifiable:

The change in the metabolism of the liver seen after pancreatectomy, and consisting of the disappearance of the ability to store glycogen and a marked increase of ketone formation, is not a direct consequence of the cessation of the internal pancreatic secretion, but a purely secondary change, conditioned by the organism's loss of carbohydrates and the consequent intense fat metabolism.

It is certainly in harmony with this view that the isolated liver from a cat treated for two days with moderate doses of phloridzin, displays qualitatively and quantitatively the same changes in the metabolism as isolated livers from pancreatectomized animals. The strongest ketone formation we have ever observed was in a liver from an animal treated with phloridzin.

That this harmonizes with the assumption I have voiced will, of course, hold good only if we accept the view that phloridzin poisoning causes a renal glycosuria which leaves the general carbohydrate metabolism unaffected. This view sounds extremely probable to me. I might mention in this connection that, in experiments with isolated rabbit livers, it is impossible to bring about any change in the utilization of glucose, even when relatively large doses of phloridzin are added to the blood (3). These experiments favor the view that, in the doses usually employed, phloridzin does not produce a general disturbance in the carbohydrate metabolism.

There is no doubt that the formation of ketone bodies in the liver, which has the character of a partial oxidation, may be compared with the partial oxidation of alcohol in the liver which I dealt with in my lecture yesterday. Like alcohol, the fatty acids presumably are not attacked, or only with difficulty, directly in the muscles. Only after a primary partial oxidation in the liver, whereby, as our experiments seem to show, the *entire* molecule is broken down to β-hydroxybutyric acid, is an oxidation in the various tissues, including the muscles, possible. Undoubtedly the classic view must be given up, according to which a formation of ketone bodies in the liver is due to a decreased

or abolished carbohydrate oxidation, as a simultaneous combustion of carbohydrates was thought necessary to secure a complete oxidation of fatty acids. In the normal cat liver with a fair glycogen content we find a respiratory quotient of 0.65. The formation of ketone bodies is only slight and the low respiratory quotient indicates a complete lack of carbohydrate oxidation. In the liver from a pancreatectomized cat we find a respiratory quotient which may be as low as 0.30. As pointed out, this low quotient is due to the pronounced formation of ketone bodies. If a rough correction of the quotient is made by subtracting the amount of oxygen used in the formation of ketone bodies according to equation II from the total amount of oxygen, the corrected quotient will be of the same magnitude as in normal livers. Setting aside the formation of ketone bodies no difference seems to exist between the oxidative metabolism proper of the normal and that of the diabetic liver, and nevertheless there is a very marked difference with regard to the intensity of formation of ketone bodies between the normal and the diabetic liver. Ketone formation may therefore be regarded as quite a normal intermediate phase in fat combustion, the intensity of which is adjusted to the intensity of that fat combustion. Even the most intense formation of ketone bodies, which we have observed in livers from pancreatectomized animals and from phloridzin-poisoned animals, can, quantitatively, hardly be described as pathological.

Some experiments by Blixenkrone-Møller are very illuminating in this connection. Blood was first perfused for two hours through a liver with pronounced formation of ketone bodies. The same blood, which in that manner had become charged with ketone bodies, was then employed for the perfusion of a hind-limb preparation. When the liver employed has a fairly intense formation of ketone bodies, the perfusion of the hind-limb can begin with a blood quantity of 250 to 300 cc. with an absolute ketone content of 200 to 250 mg. If this blood is perfused through a hind-limb preparation for 1½ to 2 hours, every trace of ketone bodies will have disappeared. The ketone bodies of course are determined both in the muscles and in the blood. This means that a quantity of muscle smaller than half of the animal's total musculature is capable of oxidizing ketone bodies just as rapidly as they are formed in an isolated liver with a very pronounced ketone

formation. Here it should be pointed out that, in the isolated liver, the ketone formation always decreases throughout the experimental period, presumably as a result of the accumulation of ketone bodies under such conditions. No doubt the formation of ketone bodies in a liver *in situ* can attain to much greater intensity.

If the experiments are performed so that the musculature of the hind-limb preparations is stimulated for a period of 15 minutes, whereby the oxygen consumption of the preparations is raised to about four times the basal consumption, all the ketone bodies will have disappeared in the course of only 20–30 minutes. Therefore it is certain that ketone bodies are utilized as a fuel in muscular exercise. It must be regarded as very improbable, not to say out of the question, that ketone bodies in an isolated hind-limb preparation are converted into carbohydrate before they are utilized.

This observation brings us back to the problem with which I dealt yesterday, for in these experiments I am inclined to see a very important proof of the assumption, that the metabolism of the aerobically working muscles is not one of carbohydrate only.

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THE USE OF THE ELECTROCARDIOGRAM IN THE DIAGNOSIS OF ADHESIVE PERICARDIO-MEDIASTINITIS

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The present study attempts to ascertain the reliability of an electrocardiographic procedure previously suggested (2) as an aid in the diagnosis of adhesive pericardio-mediastinitis. This condition, usually the result of tuberculosis or rheumatism, may be defined as the fusion of the visceral and parietal pericardia, together with adhesions binding the heart to the surrounding structures. The electrocardiographic test is based on the knowledge (3) that change in the configuration of the electrocardiogram occurs when the normal, reclining, subject is turned from side to side. This procedure, by rotating the heart, shifts the electrical axis and thus alters the form of the QRS complex.

Dieuaide (2), in 1925, published from this laboratory a report concerning the limiting effect of pericardial adhesions on the axis shift of the electrocardiogram. Included in his study were the electrocardiographic and post-mortem findings of twelve cases in which fixation of the cardiac apex was suggested by clinical signs. The correlation between the electrocardiographic and post-mortem data was shown by the following: (a) Eight cases with marked change in the form of the electrocardiogram gave no post-mortem evidence of pericardial adhesions; (b) Two cases with very slight change in the form of the electrocardiogram showed moderate involvement of the pericardium and mediastinum by adhesions; (c) Two cases with no change in the form of the electrocardiogram had, at autopsy, dense adhesions involving the pericardium and mediastinum. Dieuaide's (2) report concludes with the suggestion that fixation of the electrical axis, as determined by the electrocardiogram, may serve as objective evidence of adhesive pericardio-mediastinitis.

Sampson and Rosenblum (4), in 1934, presented a similar study of five cases of this condition verified at autopsy in four instances and at operation in one. Their results did not confirm the findings of Dieuaide, since in 4 of the 5 cases studied the shift of the electrical axis was well within normal limits. They therefore concluded that the absence of shift of the electrical axis was not specifically indicative of adhesive pericardio-mediastinitis.

METHOD OF STUDY

From the records of the Johns Hopkins Hospital since the year 1925 there were obtained 38 cases with electrocardiographic "axis shift" studies and post-mortem data. The analysis of this material is shown in Table I.

The sources of error, limiting the accuracy of the results, are three: First, the absence of a criterion for "normal" change in contour of the electrocardiogram when the reclining subject is turned from the right lateral to the left lateral position. Sampson and Rosenblum (4) attempted to solve this problem by calculating the electrical axis after the method of Carter, Richter and Greene (1). By this procedure the degree of axis shift equals the numerical difference in the values of the electrical axes obtained with the patients in the right and left lateral positions, respectively. In order to have a standard for comparison they studied the degree of axis shift in 19 presumably normal subjects. Of this group all but two had an axis shift of more than 10°. The two remaining subjects had a shift of 5° and 7°, respectively. In criticism of the above method Dieuaide (2) emphasized the fact that unless Leads I and III are taken simultaneously, so that points occurring at the same instant in the two leads may be readily compared, the value for the electrical axis thus obtained is not above question. Although determinations of the electrical axis and degree of shift were made in all cases, the present plan has been to follow the method of Dieuaide. "Fixation" of the electrical axis was determined by careful inspection of the record and limited to those electrocardiograms showing no appreciable change in the height of the QRS complex when the patient was turned from the right lateral to the left lateral position. In an attempt to create some sort of numerical standard, fixation was considered present if

TABLE I

CASE	HEART SIZE*	FIXATION OF EKG ON INSPECTION	AXIS SHIFT	HEART WEIGHT	ADHESIVE PERICARDIOMEDIASTINITIS	TIME BETWEEN EKG AND AUTOPSY	REMARKS
				Group I			
	per cent			*grams*		*months*	
H. H.		0	3°	550	+	11	Clinical impression of adherent pericardium confirmed at autopsy.
H. W.	69	0	30°	530	+	4½	Clinical impression of adherent pericardium confirmed at autopsy.
E. G.		0	30°	690	+	18½	Adherent pericardium not suspected clinically.
J. F.	60	0	18°	890	+	5½	Clinical impression of adherent pericardium confirmed at autopsy.
O. C.		0	17°	640	+	½	Clinical impression of adherent pericardium confirmed at autopsy.
W. M.	60	+	11°	550	+	1½	Pulmonary fibrosis. Adherent pericardium not suspected clinically.
M. B.	70	+	5°	820	+	23	Clinical impression of adherent pericardium confirmed at autopsy.
				Group II			
I. W.		+	10°	680	0	½	A few delicate pleuro-pericardial adhesions. No adhesions between pericardial layers.
A. H.	49	+	6°	425	0	½	Rheumatic heart disease. No pericardial adhesions.
B. N.	54	+	6°	480	0	9½	No pericardial adhesions.
				Group III			
L. F.	57	0	5°	600	0	1	Subacute bacterial endocarditis. No pericardial adhesions.
E. P.		0	9°	300	0	26	Hypertension. Amyloid disease.
C. M.	62	0	11°	470	0	3½	Rheumatic heart. No pericardial adhesions.
W. L.	67	0	13°	530	0	1	Subacute bacterial endocarditis.
F. B.	70	0	13°	430	0	½	Hypertensive heart disease.

* The heart size was determined by measurement of a teleoroentgenogram. The percentage was obtained by the following calculation: $\frac{\text{total cardiac diameter}}{\text{total thoracic diameter}} \times 100$. 50% was considered to be the upper limit of normal.

TABLE I—*Concluded*

CASE	HEART SIZE*	FIXATION OF EKG ON INSPECTION	AXIS SHIFT	HEART WEIGHT	ADHESIVE PERICARDIOMEDIASTINITIS	TIME BETWEEN EKG AND AUTOPSY	REMARKS
				Group III—*Continued*			
	per cent			*grams*		*months*	
O. M.	56	0	13°	700	0	18½	Pericarditis without adhesions to surrounding structures.
C. G.	71	0	14°		0	2¼	Tuberculous pericarditis. Pleuropericardial adhesions. Pericardial layers separated by fluid.
C. S.	56	0	16°	560	0	1	Rheumatic heart disease.
L. B.	63	0	17°	480	0	2	Rheumatic heart disease. Bacterial endocarditis.
S. S.	53	0	18°	300	0	17	Pericarditis calculosa. No pleural-pericardial adhesion.
R. B.	70	0	21°	550	0	1	No pericardial adhesions. Adherent pericardium diagnosed clinically.
W. J.		0	22°	680	0	⅔	Pleuro-pericardial adhesions. Two layers of pericardium not adherent.
A. S.	73	0	23°	400	0	1	Rheumatic heart disease.
M. H.		0	24°	720	0	1¼	Rheumatic heart disease. Bacterial endocarditis.
J. D.	56	0	25°	650	0	9½	Cardiac hypertrophy.
J. B.	52	0	27°	300	0	2	Rheumatic heart disease. Bacterial endocarditis.
J. W.	67	0	30°	570	0	12	No adhesions. Question of luetic myocarditis.
M. T.	61	0	32°	900	0	66	Luetic aortic insufficiency.
G. K.	57	0	32°	290	0	⅛	Bacterial endocarditis—gonococcus.
H. O.	69	0	34°	700	0	48	Cardiac enlargement.
M. R.	70	0	35°	470	0	20	Rheumatic heart disease.
C. D.	67	0	37°	670	0	62½	Rheumatic heart disease.
M. P.	78	0	37°	625	0	16	Hypertensive heart disease.
K. K.	42	0	40°	260	0	3½	Cirrhosis of liver. No cardiac disease.
B. W.	65	0	47°	580	0	10½	Rheumatic heart disease.
E. V.	63	0	70°	540	0	2	Rheumatic heart disease. Hypertension.
J. G.	69	0	85°	500	0	8½	Hypertension. Question of luetic myocarditis.
D. W.	72	0	93°	520	0	½	Acute pericarditis. No pleuro-pericardial adhesions.

the difference in the heights of the greatest deflection in Lead I was less than 2 mm.

The second source of error in the present study is the difficulty of evaluating the degree of cardiac fixation from the description of the pericardial adhesions given in the autopsy protocols. It may be emphasized at this point that only those cases showing adhesion of the two pericardial layers, as well as restricting adhesions between the parietal pericardium and surrounding structures, were classified as adhesive pericardio-mediastinitis.

Finally, there is the error involving the elapsed time between the electrocardiographic and post-mortem examinations. If this interval were sufficiently great, significant adhesions, absent when the electrocardiogram was taken, might be found at autopsy. Of the five cases with post-mortem evidence of adherent pericardio-mediastinitis failing to show "fixation" of the electrocardiogram, the interval between electrocardiographic and post-mortem examinations varied between five days and eleven months. In these five cases, therefore, it is possible that adhesions found at autopsy were not present at the time of the electrocardiographic test and that the discrepancy between the electrocardiographic and post-mortem findings was apparent rather than real. It should be noted, however, that clinical evidence of fixation of the cardiac apex was present in each instance at the time of the electrocardiographic examination.

DISCUSSION OF RESULTS

Of the 38 cases studied, 7 (Table I, Group I) showed post-mortem evidence of adhesive pericardio-mediastinitis. In only 2 (W. M., M. B.) of the 7 did the electrocardiogram fail to show appreciable change in the size of the QRS complex as the result of the test (Fig. I). In the remaining 5 cases the change in height of the greatest deflection in Lead I was greater than 2 mm. (Fig. II). Three cases (Table I, Group II) without post-mortem evidence of adhesive pericardio-mediastinitis showed "fixation" of the QRS complex (Fig. III).

Of the above seven cases, the diagnosis made from physical signs before death was correct in five instances, while the electrocardiographic test was positive in only two. In addition, a positive result was obtained from the test in three instances in which no adhesive pericardio-mediastinitis was found at autopsy.

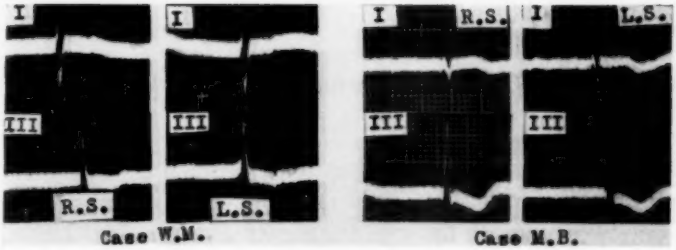

FIG. I. CASES OF ADHERENT PERICARDIUM SHOWING FIXATION OF ELECTRICAL AXIS

R.S. = right lateral position. L.S. = left lateral position.

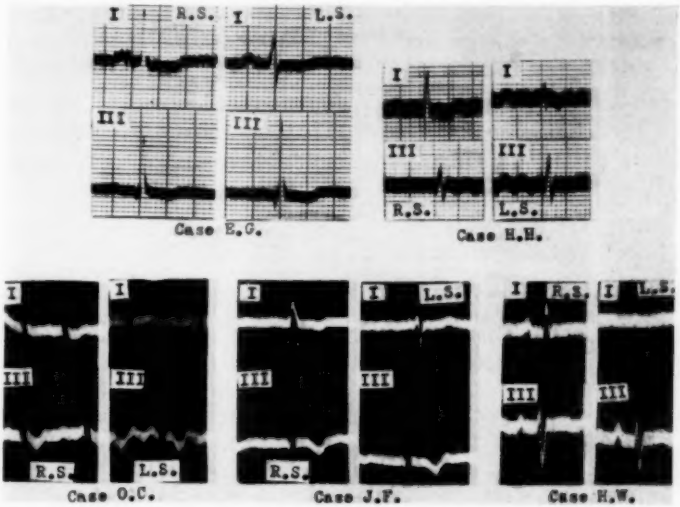

FIG. II. CASES OF ADHERENT PERICARDIUM SHOWING NORMAL AXIS SHIFT

R.S. = right lateral position. L.S. = left lateral position.

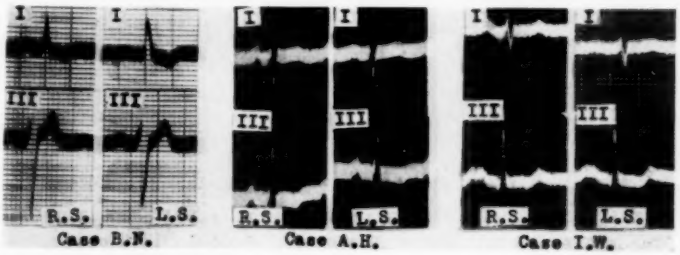

FIG. III. CASES SHOWING FIXATION OF ELECTRICAL AXIS AND NO POST-MORTEM EVIDENCE OF ADHERENT PERICARDIUM

R.S. = right lateral position L.S. = left lateral position.

These results, as do those of Sampson and Rosenblum (4), fail to confirm the earlier findings of Dieuaide. It would appear, as the result of the test, that conspicuous change in the form of the QRS complex may occur in the presence of extensive adhesive pericardio-mediastinitis. Conversely, the QRS complex may remain unaltered in the absence of pericardial adhesions.

CONCLUSIONS

An analysis has been made of 38 cases in which shift of the electrical axis was studied during life and the hearts examined at autopsy. No constant relationship was found between the fixation of the electrocardiogram and the presence of restricting pericardial adhesions. It is, therefore, concluded that the electrocardiographic test for "axis shift" is not of value in the ante-mortem diagnosis of adhesive pericardio-mediastinitis.

Note: The author is indebted to Drs. E. Cowles Andrus and Francis R. Dieuaide for helpful advice and criticism.

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THE INFLUENCE OF TONSILLECTOMY UPON THE COURSE OF RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

A Study of 108 Cases

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Although the etiological agent in rheumatic fever is still unknown, the portal of entry of the virus is considered by many to be in the upper respiratory tract. This belief is based upon observations of the frequency with which infections in the nose and throat antedate rheumatic manifestations. Tonsillectomy and adenoidectomy are frequently employed in the treatment of rheumatic fever, not as curative measures, but rather to reduce the incidence of recrudescences or to lessen in some way the severity of the infection. But in spite of the numerous publications on the subject, no unanimity of opinion exists as to the influence of tonsillectomy upon the course of rheumatic fever; it is well known, however, that individuals who have had their tonsils and adenoids removed may subsequently acquire the disease.

The following observations are reported not only because they throw some light upon the influence of tonsillectomy upon the course of rheumatic fever, but also because they contribute data concerning the course of the disease over a long period of time in the community of Baltimore. The acute rheumatic manifestations are not as outspoken or as easily recognized in Baltimore as they are in certain other localities and, for this reason, recrudescences of the disease may be overlooked by both patient and physician. Longcope (11) reviewed the histories of a number of cases seen in The Johns Hopkins Hospital and reported that rheumatic fever is practically as common here as it is in the hospitals of Boston, but that the character of the disease is not precisely the same. This difference in symptomatology renders it

difficult to compare our results with those reported in studies of cases observed elsewhere.

CASE MATERIAL

In 1917 Crowe and his associates (4) reported a study of the relationship of tonsillar and nasopharyngeal infections to systemic disorders such as nephritis, arthritis, tuberculosis and rheumatic fever. The therapeutic advantages of tonsillectomy and adenoidectomy in such conditions were discussed. Conclusions were based upon the study of a large group of patients followed for a relatively short period, namely, from 3 to 10 years. Of this group 55 cases were thought to fall into the broad field of rheumatic fever. During the year 1935, 29 of the original 55 patients coöperated in returning to permit further studies. In addition there are available adequate records of 7 other patients, thus bringing the total number of the original group followed to 36. The information about the remaining 19 patients is too meager to include in this series.

To the above 36 cases, we have added 72, bringing the total number to 108 cases. The additional patients were taken in sequence from the records of The Johns Hopkins Hospital. They were all individuals with rheumatic fever who had been subjected to tonsillectomy and adenoidectomy. Many patients operated upon during the period covered by this series of cases (1910 to 1924) had to be eliminated from the investigation either because the original examinations had not been carried out in sufficient detail, or because the patients could not be located in the year 1935.[1] Otherwise, no selection was exercised in the choice of cases.

Of the total 108 cases, 90 have been followed from 10 to 23 years, and 18 from 1 to 10 years. The follow-up period of 16 of the latter group was terminated by the death of the patients. The average age of the 86 living patients was 26.4 years in 1935.

RESULTS

A general summary of the observations is illustrated in Chart 1. The cases have been divided into 5 groups depending upon the length of the postoperative period of observation, namely, 1 to 5 years, 6 to 10 years, 11 to 15 years, 16 to 20 years and 21 to 23 years.

[1] Total number of cases investigated	216
Patients examined in 1935	86
Patients living; refused to be examined	18
Patients living; refused to answer letters	40
Patients not located; status unknown	29
Patients probably dead—inadequate information	21
Patients dead—adequate information	22

In the group of those followed for 1 to 5 years there were 11 patients. All 11 had recrudescences, 3 single and 8 multiple. All the patients in this group are dead. The cause of death in every case was rheumatic heart disease.

The group followed for 6 to 10 years included 15 patients, 8 of whom had recrudescences, 4 single and 4 multiple. Of this group 5 patients died, two deaths

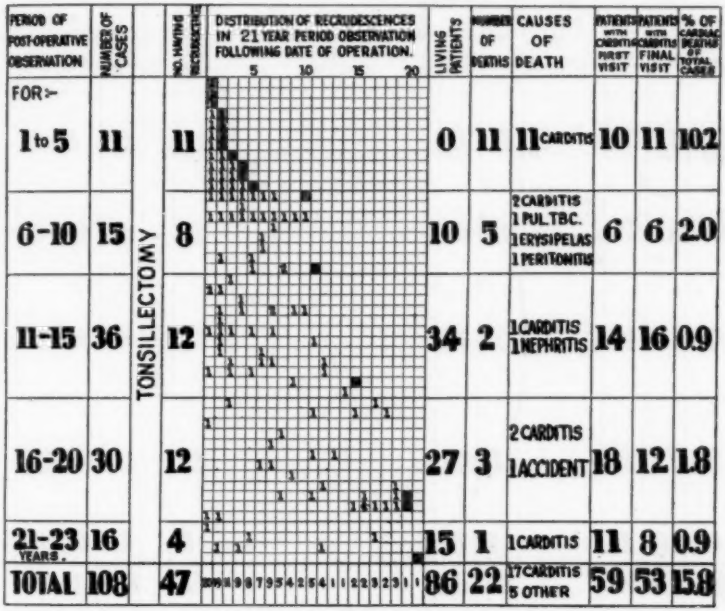
PERIOD OF POST-OPERATIVE OBSERVATION
NUMBER OF CASES
TONSILLECTOMY
NO. HAVING RECRUDESCENCE
DISTRIBUTION OF RECRUDESCENCES IN 21 YEAR PERIOD OBSERVATION FOLLOWING DATE OF OPERATION.
5
10
15
20
LIVING PATIENTS
NUMBER OF DEATHS
CAUSES OF DEATH
PATIENTS WITH CARDITIS FIRST VISIT
PATIENTS WITH CARDITIS FINAL VISIT
% OF CARDIAC DEATHS OF TOTAL CASES
FOR>
1 to 5 11 11 0 11 11 CARDITIS 10 11 10.2
6-10 15 8 10 5 2 CARDITIS 1 PUL. TBC. 1 ERYSIPELAS 1 PERITONITIS 6 6 2.0
11-15 36 12 34 2 1 CARDITIS 1 NEPHRITIS 14 16 0.9
16-20 30 12 27 3 2 CARDITIS 1 ACCIDENT 18 12 1.8
21-23 YEARS. 16 4 15 1 1 CARDITIS 11 8 0.9
TOTAL 108 47 86 22 17 CARDITIS 5 OTHER 59 53 15.8

CHART 1. SUMMARY OF 108 PATIENTS

The cross-ruled section of the chart includes only the 47 patients in whom recrudescences occurred. The shaded squares indicate deaths. All of these deaths occurred in patients having rheumatic heart disease. The word "carditis" is used for the sake of brevity, it signifies rheumatic cardiac disease in general, not necessarily acute or subacute cardiac disease.

being attributable to rheumatic heart disease, one to tuberculosis, one to erysipelas and one to peritonitis following an appendectomy.

Thirty-six patients were followed for 11 to 15 years. 12 of these had recrudescences, 3 being single and 9 multiple. There were two deaths in this group, one from rheumatic heart disease and one from nephritis.

Thirty patients were followed for 16 to 20 years and of these 12 had recrudes-

cences. Of the 12, 4 had single and 8 had multiple recrudescences. In this group there were 3 deaths, 2 from heart disease and 1 from drowning.

Sixteen patients were followed for 21 to 23 years. In this group 4 had recrudescences—2 with single and 2 with multiple attacks. The single death in this group was due to acute rheumatic fever with pancarditis.

It has been pointed out repeatedly that recrudescences of rheumatic fever are more likely to develop within the first 5 years after the initial manifestations of the disease than at any time subsequently. This fact, stressed by Kaiser (10), in a recent communication, is graphically illustrated in Chart 1. But in Chart 1 it may also be seen that those patients who live more than 5 years after tonsillectomy have as many recrudescences after the 5-year period as during it. In fact recrudescences may be observed at any time provided the patient is kept under

TABLE I

Grouping of patients according to status at time of operation

GROUP	TYPE OF RHEUMATIC DISEASE	NUMBER OF CASES
I	Polyarthritis	36
II	Chorea	46
III	Combined	16
IV	Carditis	10
Total		108

observation for a sufficient number of years. The history of one of the patients in the 21- to the 23-year group illustrates this point very strikingly. Prior to operation she had had repeated attacks of acute rheumatic fever resulting in well defined mitral stenosis and insufficiency. There were no recrudescences until 21 years after operation but at that time she had an attack of acute rheumatic fever during which she died. It is possible that such an occurrence was due to reinfection rather than to a recrudescence of the original infection, but it illustrates the fact that acute rheumatic fever may recur after a lapse of many years.

In order to study the influence of tonsillectomy and adenoidectomy upon the various manifestations of rheumatic fever, the cases have been grouped according to the type of rheumatic disease which existed prior to operation (Table I). In this table 16 patients have been

placed in what has been designated a "combined group" (Group III). All of these patients had both polyarthritis and chorea before operation. Ten patients who gave no history of antecedent rheumatic infection, but who had both acute rheumatic polyarthritis and well defined rheumatic carditis at the time of their first hospital admission, have been placed in the "carditis group" (Group IV).

The incidence of recrudescences in the total 108 cases and in the 4 groups is shown in Chart 2.

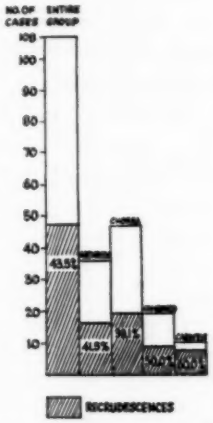

CHART 2. INCIDENCE OF RECRUDESCENCES IN THE VARIOUS GROUPS OF PATIENTS

In previous studies of rheumatic disease evidence has been brought forth to show that permanent cardiac damage is more likely to occur after repeated attacks of rheumatic fever than after a single attack. This has been studied in our patients by a further division of the cases into two categories: (1) those who gave a history of only 1 attack of rheumatic disease prior to tonsillectomy and (2) those who had had multiple attacks prior to operation. The carditis group has not been so divided, although it is believed that many of these patients had had some form of rheumatic disease prior to the first recognized attack. It is of interest and somewhat contrary to general experience that 7 patients in the combined group had single simultaneous attacks of polyarthritis and chorea at the onset of their illness. In Table II

it may be seen that irrespective of the number of rheumatic episodes prior to operation there were recrudescences subsequent to operation in less than one-half of the cases.

Postoperative course of patients who had suffered from a single attack of rheumatic disease prior to operation. (a) Of the 19 patients in the arthritis group, all but 3 were subjected to tonsillectomy within a year after the initial rheumatic episode. In the 3 exceptions the attacks occurred at earlier dates: in one, 3 years before, in another, 5 years

TABLE II

Recrudescences of rheumatic disease and deaths

	SINGLE ATTACKS BEFORE OPERATION				MULTIPLE ATTACKS BEFORE OPERATION			
			Deaths				Deaths	
	Recrudescences	No recrudescences	Recrudescences	No recrudescences	Recrudescences	No recrudescences	Recrudescences	No recrudescences
Polyarthritis	8	11	4	3	7	10	2	1
Chorea	13	18	2	1	5	10	2	0
Combined	4	3	3	0	4	5	0	0
Carditis	6	4	4	0				

	Deaths		Deaths	
	Recrudescences	No recrudescences	Recrudescences	No recrudescences
Polyarthritis	4 Cardiac	1 Nephritis 1 Erysipelas 1 Tuberculosis	2 Cardiac	1 Drowned
Chorea	2 Cardiac	1 Peritonitis	2 Cardiac	0
Combined	3 Cardiac	0	0	0
Carditis	4 Cardiac	0		

before, and in a third, 25 years before. Following operation 8 of the 19 patients had recrudescences which were in all instances in the form of polyarthritis. In 4 there were multiple recrudescences occurring from 1 to 20 years after operation and 4 had single recrudescences. There were 4 deaths in the group. One patient whose single recrudescence occurred 9 years after operation died of subacute bacterial endocarditis $14\frac{2}{3}$ years after operation. The other 3 patients, 2 of whom had had multiple recrudescences, died of rheumatic heart disease. Of these 3 patients, 1 died 20 years and another 3 years

after operation. The third patient died within a year after the operation during the first recrudescence.

There were 11 patients in the arthritis group who did not have recrudescences and, of these, 3 died. None of the deaths could be attributed directly to rheumatic infection. One patient died of erysipelas 8 years after operation, another of pulmonary tuberculosis 5 years after operation, and the third, of chronic glomerular nephritis 13 years after operation.

(b) In the chorea group, of the 31 patients who had had a single attack prior to tonsillectomy 13 had recrudescences. Five patients had a single recrudescence occurring between 1 and 6 years after operation. In all but 4 instances the recrudescences were in the form of chorea. One patient had multiple attacks of polyarthritis during which rheumatic heart disease appeared. Three patients had several attacks of chorea alternating with attacks of acute polyarthritis. Two patients in the entire group died; one of rheumatic heart disease, and the other of rheumatic heart disease complicated by pneumonia.

In 18 patients no recrudescences occurred. The one death in this group was due to peritonitis resulting from a ruptured appendix.

In the chorea group, all the tonsillectomies were performed within a year of the initial attack with 2 exceptions, and in these the original single attacks of chorea had occurred 3 years before the operation.

(c) In 4 patients in the combined group a single recrudescence occurred in the form of a simultaneous attack of chorea and polyarthritis. Of the 3 patients who died all had rheumatic heart disease, although the immediate cause of death of one of them was pneumonia. The 3 patients who did not have recrudescences are all living and well.

(d) In the carditis group it is difficult to state whether there were single or multiple attacks of rheumatic fever before operation. Of the 10 patients in this group 6 had rheumatic manifestations subsequent to operation. In all instances recrudescences were in the form of polyarthritis which came in multiple attacks. Of the 6 patients 4 died within 3 years and all deaths were directly attributable to rheumatic heart disease.

Postoperative Course of Patients Who Had Had Multiple Attacks of Rheumatic Disease Prior to Operation. (a) Of the 17 patients in the

arthritis group 7 had recrudescences and 10 did not. There were two deaths in the former group; 1 due to rheumatic heart disease, the other to rheumatic heart disease complicated by acute yellow atrophy of the liver. There was 1 death in the latter group but this was due to drowning. Recrudescences were in the form of polyarthritis and, except in 2 patients, were multiple. They occurred between 1 and 21 years after operation.

(b) Of the 15 patients in the chorea group 5 had recrudescences which were in all instances in the form of chorea. Except in 1 of the 5 patients, the recrudescences were multiple. All of them occurred between 1 and 22 years after operation. Two of the patients who had recrudescences died, both as a result of rheumatic heart disease. There were no deaths among those who did not have recrudescences.

(c) Of the 9 patients in the combined group 4 had recrudescences, which were in all instances multiple. There were no deaths in this group.

(d) As far as is known, none of the patients in the carditis group had had multiple recrudescences before operation.

The Relationship of Persistent Nasopharyngeal Infections to Recrudescences. Of the 86 patients who returned for examination 29 were found to have foci of infection in the nasopharynx. Of the returning patients 25 had had recrudescences of rheumatic fever and 17 of this group were among those with foci of infection in the nasopharynx. The incidence of persistent infection of the nasopharynx was, therefore, considerably higher in the patients who had recrudescences than in the entire group.

The Influence of Recrudescences Upon the Appearance and Progression of Rheumatic Heart Disease. Thirty-one patients who had recrudescences subsequent to tonsillectomy were considered to have rheumatic heart disease of some degree at the time of the initial examination. In 6 of these patients the signs of cardiac involvement subsequently disappeared. Twenty-five of them had either died of rheumatic heart disease or were found to have well defined rheumatic heart disease at the time of our final examination. The patients have been divided arbitrarily into 2 groups (a) progressive, and (b) stationary. The term "progressive" has been applied to those patients who showed an increase in the extent of cardiac involvement together with symptoms, or with both signs and symptoms of heart

failure. The term "stationary" has been applied to those patients whose cardiac lesion showed no change after the original examination. Of the 25 patients 16 were in the "progressive" and 9 in the "stationary" group. Of the former group 13 patients had died, while 3 were found to have evidences of extensive cardiac involvement with both signs and symptoms of myocardial insufficiency.

Six patients who subsequently had recrudescences gave no evidence of cardiac disease at the time of the original examination. Four of these, after a number of recrudescences, died of rheumatic heart disease, and an additional patient at our last examination was found to have cardiac involvement.

Twenty-eight patients who had no recrudescences subsequent to operation were considered to have rheumatic heart disease at the time of the original examination. In the recent reëxaminations only 16 of this group showed any evidences of cardiac involvement. Six patients had signs which were identical with those observed at the original examination, while 10 had more extensive cardiac involvement. Only 4 of the latter group had had symptoms and only 1 of these had had signs of myocardial insufficiency. There were no deaths in this group.

Thirty-three patients who did not have recrudescences after tonsillectomy showed no signs or symptoms of cardiac involvement at the time of the original examination. None of the patients in this group died of heart disease, and in a recent examination none showed signs of cardiac involvement except one who had mitral stenosis without symptoms or signs of myocardial insufficiency.

Coombs (3) states that the signs of cardiac involvement disappear in about 30 per cent of all cases of rheumatic fever. The disappearance of signs in 30.5 per cent of our cases (18 out of 59) is in keeping with Coombs' figure. Bland, Jones and White (1) have considered Coombs' figure to be too high.

DISCUSSION

In this study an effort has been made to determine the effect of tonsillectomy and adenoidectomy upon (1) the incidence of recrudescences of rheumatic fever, and (2) the appearance or progression of rheumatic heart disease in known rheumatic subjects.

Recrudescences subsequent to operation were recognized in 47

individuals, that is, in 43.5 per cent of all cases. The latter figure is somewhat lower than the incidence of recrudescences reported from other localities. It should be emphasized, to reiterate what was said in the opening paragraphs of this paper, that studies carried out in different localities are not strictly comparable. Swift (15) reports an incidence of recrudescences as high as 60 per cent. Schlesinger (13), in England, reports an incidence of 58 per cent. Howard and Mills (7), in Canada, report recrudescences in 67 per cent of cases. Kaiser (10), in Rochester, New York, states that there are recrudescences in over 50 per cent of the cases and a similar figure is given in a recent report from Virginia (5). These percentages were obtained from studies of large series of cases in which the influence of tonsillectomy was not dealt with. Whether the lower incidence of recrudescences in our cases was due to the removal of the tonsils and adenoids cannot be satisfactorily determined because the series is too small to permit statistical deductions. Furthermore, it should be noted that the operation was not in all cases coincident with the initial rheumatic infection. Some of the patients had had recrudescences prior to operation.

The incidence of cardiac involvement in our arthritis group was 47.1 per cent. If the combined group is added to this, the incidence in all patients with polyarthritis increases to 51.9 per cent. In the chorea group the incidence of cardiac involvement was 26.1 per cent. The general consensus of opinion regarding the influence of tonsillectomy upon the incidence of rheumatic heart disease is in accord with that of Hunt and Osman (8), who believed that the operation had little or no beneficial effect. Reports dealing with this subject generally place the occurrence of heart disease after rheumatic fever at about 60 per cent. Some individual reports give a much higher incidence. Coombs (3) reports that 75 per cent of rheumatic subjects who exhibit joint pains develop rheumatic heart disease, whereas the incidence is only 44 per cent among patients in whom chorea is the only rheumatic manifestation. Mackie (12) states that there is little difference between the arthritis and chorea groups. He gives the incidence of cardiac involvement as 68.3 per cent irrespective of age and as 78.2 per cent when the first attack occurs between 10 and 12 years of age. Howard and Mills (7) give the incidence of cardiac disease as 68

per cent. Strong (14), in a large series, gives the incidence as 38 per cent in those exhibiting joint manifestations, and as 45 per cent in those having chorea. This is not in accord with the findings of other observers in that the incidence of cardiac involvement is greater among those with chorea than among those with joint manifestations. Houston (6), in New Orleans, comments upon the mild character of the arthritis in that community and gives the incidence of cardiac disease as 51.1 per cent. In general, a review of the literature shows that the incidence of rheumatic heart disease in cases of rheumatic polyarthritis is approximately 60 to 70 per cent, while the incidence in cases of chorea is approximately 50 per cent. In some reports there is a wide divergence from these figures. For example, Jones and Bland (9), using the term "pure chorea" to denote those patients in

TABLE III

	CARDIAC INVOLVEMENT AT TIME OF OPERATION		NO CARDIAC INVOLVEMENT AT TIME OF OPERATION	
	Recrudescences	No recrudescences	Recrudescences	No recrudescences
Total cases	31	28	16	33
Died. Rheumatic heart disease	13	0	4	0
Living. Rheumatic heart disease	13	16	1	1

whom the original and subsequent rheumatic manifestation is chorea, report the incidence of cardiac manifestations in such patients as 3 per cent. In the Baltimore community Thayer (16) gives figures which indicate that the incidence of cardiac involvement is 75 per cent in patients with rheumatic polyarthritis. He states that the incidence in cases of chorea is 25 per cent among those treated in the Out-Patient Department but is over 50 per cent among patients admitted to the hospital wards for treatment.

In considering the effect of tonsillectomy it is of interest that the incidence of cardiac involvement in our patients was considerably lower than that observed by Thayer (16) in the same community. The lower incidence holds, whether one considers the entire group or the individual groups having polyarthritis or chorea. The statistical significance of this difference is questionable. Nevertheless, it is encouraging to discover that among the 49 patients not having cardiac

involvement at the time of tonsillectomy (see Table III) only 6 (12.2%) developed rheumatic heart disease subsequent to operation. In this group of patients there appear to be sound reasons for recommending tonsillectomy.

SUMMARY

1. One hundred and eight patients subjected to tonsillectomy and adenoidectomy because of rheumatic fever, between 1910 to 1924, were reinvestigated in 1935.

2. Following operation recrudescences of acute rheumatic manifestations occurred in 43.5 per cent of the patients.

3. Recrudescences were common in the first 5 years after operation. Most of the patients who had repeated recrudescences during this period died of rheumatic heart disease.

4. Patients living for more than 5 years after operation had almost as many recrudescences after the 5-year period as during it.

5. Evidence of continued nasopharyngeal infection was prominent in the patients having recrudescences.

6. Chorea alone was less often followed by severe rheumatic heart disease than was polyarthritis alone.

7. Rheumatic heart disease occurred more frequently in those having recrudescences, and deaths from rheumatic heart disease occurred only in this group of patients.

8. The total incidence of rheumatic heart disease was relatively but perhaps not significantly low.

9. Since rheumatic heart disease developed in only 6 of the 49 rheumatic patients not having cardiac involvement at the time of operation, it is concluded that tonsillectomy and adenoidectomy are to be recommended in the treatment of rheumatic fever.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Personnel Policies in Public Health Nursing. By MARIAN G. RANDALL. 170 pp. $2.00. (*The Macmillan Company, New York, 1937.*)

Public health programs vary in different localities but everywhere they are affected by the type of personnel in both official and non-official services. Although non-official agencies have followed fairly closely the standards recommended by the N. O. P. H. N., up to the present time very little has been known about the policy of official agencies regarding personnel selection. Since well over one half of the public health nurses in the field are employed by official agencies and because of an increasing demand for well qualified personnel, the advantage of having up-to-the-minute information on the subject is readily seen.

Among the good features of the book are the concisely stated summary and recommendations, 14 tables for ready reference, a suggested classification of 4 grades of work needed to carry out various types of health programs; several chapters on conditions of work which would serve to attract well prepared nurses, and a brief history of civil service in the United States.

It is interesting to learn that a large proportion of official agencies with nursing supervisors have higher requirements than the present minimum standards recommended by the N. O. P. H. N., whereas a number of smaller agencies without nursing supervisors have educational requirements below the recognized standard. Most encouraging are the striking indications of change for the better in the educational qualifications of public health nurses employed by official agencies. There is also evidence to show that the number of qualified nurses is not yet equal to the demand for them. Much is still to be desired in the matter of health programs, conditions of work and salaries before the best prepared public health nurses will be attracted by the available positions.

The author purposes to follow this study with a report, presenting in detail material obtained through personal interviews and observations of work. Information regarding the application of the health program will be eagerly awaited by agency executives.

Health officials should find this book extremely valuable. It is recommended for study by board members and executives of both official and non-official agencies, as well as for use in staff education programs.

M. A.

On a New Gland in Man and Several Mammals (Glandulae Parathyreoideae). By IVAR SANDSTRÖM. [Upsala Läkareförenings Förhandlingar, 1879–80, **15,**

441–471.] Translated by Carl M. Seipel. Edited by Charlotte H. Peters and J. F. Fulton. With biographical notes by Professor J. August Hammar. 44 pp. Illus. $1.00. (*The Johns Hopkins Press, Baltimore, 1938.*)

It is of great historical interest to have available the paper of Sandström upon his original discovery of the parathyroid. Although it is now well known that he was the first to recognise these glands, his paper was published in 1880 only in the proceedings of a scientific association in Sweden and was quite overlooked until Gley, in 1891, rediscovered these structures and drew attention to his paper. Although Sandström found only two parathyroids in some animals, and said nothing about their softer consistency, and although the technical methods in use then were not accurate enough to allow him to distinguish cells of different types in these glands, still his descriptions are remarkably good and detailed. His discussion of differentiation from the thyroid, and especially from small accessory thyroids, is clear and he gives much space to the study of their embryological relations.

Since his time, of course, much has been done. It has been found that tetany follows their destruction—Dr. Halsted was one of the first to attempt their replacement by an extract or by implantation of living gland. Their importance in the metabolism of calcium has been determined and a more accurate method of making a potent extract, parathormone, found, but still there is much to learn of their relation to the whole endocrine system.

W. G. M.

Methods of Treatment. By LOGAN CLENDENING. 6th ed. 879 pp. Illus. $10.00. (*C. V. Mosby Company, St. Louis, 1937.*)

The sixth edition of this excellent textbook does not differ materially in its general plan from previous editions. It includes an adequate survey of the more important drugs, a full description of the principal therapeutic procedures, and a discussion of their application to the treatment of the various diseases.

The book has been brought up to date, except as limited by the conservatism of the author in accepting new procedures of uncertain efficacy. He has included, for example, the use of mandelic acid, of protamine zinc insulin and, though somewhat briefly, of sulfanilamide. He has accepted artificial pneumothorax as a useful procedure in tuberculosis, but does not endorse antipneumococcus serum for the treatment of pneumonia.

The book should be useful both to students and practitioners.

P. W. C.

A Textbook of Materia Medica, Pharmacology and Therapeutics. By A. S. BLUMGARTEN. 7th edition, completely revised. 845 pp. Illus. $3.00. (*The Macmillan Company, New York, 1937.*)

This volume is written as a textbook for nurses, "to teach the nurse to observe the effect of drugs on patients, to recognize the earliest toxic symptoms, to under-

stand the objectives of the physician in prescribing a remedy, and to administer a remedy in such a manner that the maximum desired effect is obtained."

The author has included a large number of drugs and other preparations, including most of the new ones, a brief description of each, the dose, and the mode of administration.

He discusses in a very elementary way the normal and pathological physiology of the organs or functions to be treated. This simplicity, however, is often carried so far as to sacrifice precision and even accuracy. Thus: "Arsenic prevents the tissues from being used up by lessening their combination with oxygen." Epinephrin "stimulates the heart by contracting the peripheral blood vessels." Barium chloride "acts like digitalis as a heart stimulant."

The book contains a large volume of useful information. The untoward or toxic effects of the drugs are well covered. It should continue to find a wide field of usefulness in the circle for which it is intended.

P. W. C.

Operative Surgery. By J. SHELTON HORSLEY and ISAAC A. BIGGER. 4th ed. 1387 pp. in 2 vols. Illus. $15.00. (*C. V. Mosby Co., St. Louis, 1937.*)

This is one of the most salutary and serviceable contributions to the literature on operative surgery that has appeared since "The Text Book on Operative Surgery," by Theodore Kocher in the early 1900's. Whether or not one agrees with the authors' various explanatory hypotheses, one cannot but admire and regard with the deepest interest the constant repetition of the suggestion that the art of operating, combined with a thorough knowledge of anatomy, while essential, must never blind the surgeon to, or allow him to be unmindful of, the correct physiological principles involved and the consequent biological effects of a surgical operation. The student of surgery is importuned constantly to recall that "Real progress in surgery lies not so much in cultivating the art of surgery and in striving after mechanical dexterity, which is important, but can be acquired in a few years, as in the study of biological principles that concern function, nutrition, metabolism and repair of tissue and in the thoughtful application of these principles to every method of surgical treatment." This plea to regard surgery in its broader aspect is most stimulating and distinguishes this treatise from other operative surgeries, which while perhaps more inclusive are mere technical encyclopedias. The large personal experience of Dr. Horsley, Senior, and his distinguished collaborators has been related in a most readable manner and the format of this textbook is excellent. It might be said, without exaggeration, that this is the only Operative Surgery published in recent years in which scholarship has been so nicely combined with critical selection of material. The authors make no attempt to be all inclusive. Their concise description of technical procedures personally experienced, together with opinions and suggestions as to the relative merits of special operations described by others, reveals penetrating thought and mature judgment. There has been no "stuffing" of the text to form an impressive but cumbersome index and bibliography. At the conclusion of each chapter succinct and relevant references provide an accessible and ready index to—the more detailed literature pertaining to each subject.

Every operating surgeon and medical student as well as those in surgical research should have these volumes on their book shelves.

W. F. R., Jr.

Physical Diagnosis: The Art and Technique of History Taking and Physical Examination of the Patient in Health and in Disease. By DON C. SUTTON, M.S., M.D. Price, $5. Pp. 495, with 306 illustrations. (*St. Louis: C. V. Mosby Company, 1937.*)

Physical diagnosis is a discipline fundamental to clinical medicine. The neophyte may find it as dull as a Latin conjugation, or as rewarding as the gift of sight to the blind. But he can no more acquire proficiency from a book alone than become a pianist by contemplating a musical score.

The author of a textbook in this field can at best, therefore, describe the background and technique, and point the way along which accumulated experience may lead. This Dr. Sutton has done, crystallising in this volume the experience of his years of teaching. The illustrations are well selected, though some, particularly the radiographs and cross sections, are not too well reproduced. They are logically presented to afford representative examples of the abnormalities which may be revealed by the methods described.

In both illustrations and text the author has commendably avoided presenting a mere catalogue of physical signs, which would reduce the study of the patient from a genuine examination to the level of a search.

E. C. A.

A Primer for Diabetic Patients. By RUSSELL M. WILDER. 6th ed. reset. 191 pages. Illus. $1.75. (*W. B. Saunders Company, Philadelphia, 1937.*)

A revision of a diabetic manual which includes protamine insulin therapy. The chapters dealing with the administration and regulation of insulin are presented very clearly. Especially noteworthy is the one on the care of the feet in diabetic patients. The complete list of food substitutions and the food nomogram should be especially valuable to the physician who has to teach his patient without the aid of a trained dietitian.

G. W. T.

Paracelsus en Hahnemann, een Renaissance der Geneeskunst. By R. A. B. OOSTERHUIS. IX + 300 pp. *Leiden, A. W. Sijthoff's Uitgeversmaatschappij N.V., 1937.*)

The author compares Hahnemann's *Homeopathy* with the "Essential Therapy" of Paracelsus. This latter term he derives from the *Volumen Paramirum* in which Paracelsus expounds the five "entia" dominating disease and the various principles of treatment, including that by specifics. Oosterhuis arrives at the conclusion that this Essential Therapy represents Paracelsus' conception of the cure by similars. The relation of Paracelsian ideas to the system of Hahnemann has been pointed out by various authors whom Oosterhuis reviews on pp. 86–106,

although Hahnemann developed his ideas independently. What characterizes Oosterhuis' approach is a thorough and objective analysis of Paracelsus as well as of Hahnemann. Although himself inclined to homeopathy, his attitude towards Hahnemann is critical; he points out differences between Hahnemann and Paracelsus and emphasizes the fact that the latter was not a systematic thinker. In this opinion the author is certainly right, and it is therefore regrettable that in his summary (p. 296) he makes the statement: "The Essential Therapy is the homeopathy of the Renaissance. Homeopathy is the Essential Therapy of modern times." As a matter of fact, the so-called Essential Therapy is but one aspect of Paracelsus' work, and generalizations like the above tend to obscure the different character of different epochs.

O. T.

The Fight for Life. By PAUL DE KRUIF. 342 pp. $3.00 (*Harcourt, Brace and Company, New York, 1938.*)

The general public is becoming increasingly more interested in the trends of modern medicine, both scientific and economic, and in his latest book Paul de Kruif has given them a lot to think about. His main theme is that, while medical science has developed great resources to prevent and cure disease, these have not been applied on any large scale because most patients cannot afford them.

To illustrate his thesis he tells of some of the work being done in different fields of medical research, such as the efforts of the Chicago Maternity Center to reduce maternal mortality, the various attempts to guard against infantile paralysis and tuberculosis, the possibilities of artificial fever therapy, the campaign against syphilis, and of the discovery, use and abuse of sulfanilamide. He emphasizes that the application of these new methods demands a high degree of vigilance and training on the part of the medical men employing them, and that this in turn tends to restrict their use because of the cost.

Although, as he shows in his chapter on the fight against pellagra in the South, there are some who take the antisocial view that it is not right or worth while to save the lives of the under-privileged, the mass of the people and the great majority of the medical profession want to see a wide application of all the advances of modern science. The conditions of medical practice being what they are, de Kruif feels the solution lies in the extension of the public health activities of the government with the removal of considerations of private profit.

S. V. L.

Med.
Dupont
9-25-39

Thomas Barnes Futcher

January 1, 1871—February 25, 1938

Dr. Futcher was born at St. Thomas, Ontario, Canada, on New Year's Day, 1871. He obtained his early education in Canada and studied medicine at the University of Toronto Medical School, graduating in 1893 with the degree of M.B. He served a year as house officer in the Toronto General Hospital, and first came to Baltimore in 1894 to act as resident physician during the summer months at a convalescent home for children at Mount Wilson.

In this capacity, he must have attracted some attention, for during the same year he was selected by Dr. Osler to act as assistant resident physician at the Johns Hopkins Hospital. Though the hospital had been in operation for some years, the medical school did not open until 1893, and Futcher, therefore, enjoyed a few years of what must have been a most stimulating and intimate association with Dr. Osler before the students came into the dispensary and hospital for their work in medicine. Indeed he did not actually start his career as a teacher until 1898, for in 1896 he went abroad for two years, studying at Gratz and at Strassburg.

Dr. Futcher returned to Baltimore in 1898 to assume the duties of Resident in Medicine, an enviable post which had become vacant through the resignation of Dr. Thayer. As Resident his position was one of great responsibility for Dr. Osler not only held him accountable for the care of all the patients on the medical service but also placed him in charge of important teaching in medicine. Dr. Futcher made rounds with the students in the wards, supervised the practical work of the clinical clerks, and conducted an admirable course in clinical microscopy. Dr. Osler was responsible for the introduction of this course as a part of the clinical instruction in medicine and was therefore intensely interested in the manner in which it was conducted. At that time the laboratories of clinical microscopy were *the* laboratories of the clinic. They stood for a remarkable innovation in the organization of an American Medical School. It was thus not only on the

wards, but also in the laboratory that Futcher came into intimate and stimulating contact with Dr. Osler, during one of the most active periods of his brilliant career. The influence of the "Chief," as Dr. Futcher always called him, had a profound and lasting effect upon this young and eager assistant, and an intimacy grew up between the older and younger man which increased as years went on, until Sir William Osler's death.

It was during Futcher's term as Resident that he acquired the affectionate nickname of "Father" which was bestowed upon him by Dr. Osler. By this title he was known to his intimates for many years.

Dr. Futcher's ability was properly recognized and consequently he was appointed to the position of Associate in Medicine in 1897, Associate Professor of Medicine in 1901, and Associate Professor of Clinical Medicine in 1914, at which time he became one of the Visiting Physicians to The Johns Hopkins Hospital. After relinquishing his post as Resident Physician in September 1901, Dr. Futcher lived with a group of friends, among whom were Dr. Henry Barton Jacobs and Dr. Harvey Cushing, in a house on Franklin Street next to the Osler home. In these surroundings, he started the practice of medicine, continuing at the same time his duties at the medical school, hospital, and dispensary where he worked and taught daily.

In 1909, Futcher married Gwendolyn Marjorie Howard, the daughter of Dr. R. Palmer Howard, the teacher whom Dr. Osler admired and held in the highest esteem. The union, naturally, brought Dr. Futcher still more intimately into the Osler household. Later, many summers were spent in visits to Oxford.

The War interrupted the routine of the hospital work and the large practice which he had so soon acquired. It was characteristic that Futcher should have joined the Canadian forces. He acted as Lieutenant-Colonel in the Army Medical Corps and was placed in charge of the Medical Division of No. 16 Canadian General Hospital at Orpington, Kent, England.

Throughout the 44 years during which Dr. Futcher was connected in many capacities with the Johns Hopkins Hospital and Medical School, he never ceased to teach and to practice medicine. His fund of information as well as his careful and precise methods commanded

the respect of all his students, while his unusual kindness and consideration endeared him not only to students but to patients, friends, and all with whom he came in contact.

Though his medical interests were wide, his attention was particularly attracted to the problems presented by diabetes and gout, on which he was an acknowledged authority, and by diseases of the endocrine glands. He wrote much upon these subjects and contributed chapters to several important systems of medicine. He was an active member not only of the local medical societies, whose meetings he attended with great regularity, but of many of the national organizations. He was one of the original members of the Interurban Clinical Club and acted for one year as its President. The Association of American Physicians always attracted his interest and he was made President of this Association in 1932. The breadth of his interest is well indicated by the fact that he was for many years an active member of the P. L. Club, and in 1932 was elected President of the Tudor and Stuart Club of the Johns Hopkins University, of which he was an original member.

Dr. Futcher's life stands as an example of conscientious devotion to professional duties, of sympathetic solicitude for patients, and of courteous thoughtfulness for a host of devoted friends. Perhaps nothing better exemplifies these attributes than the manner of his death. Though he had had premonitions of impending trouble, he spent the morning of his last day arranging for a clinic and visiting patients at the hospital. His fatal illness came a moment after he had left the room of a patient.

Dr. Futcher was a gentleman and a physician of rare nature and of perfect integrity. With his death there passes from the Hospital and Medical School a personality whose influence cannot be estimated by a mere recitation of professional attainments. This loss brings great sorrow to his friends and his associates.

W. T. L.

PROGNOSIS IN CASES OF TUBERCULOSIS OF THE TONSILS, ADENOIDS AND CERVICAL LYMPH NODES

A Study of Patients Followed for 11 to 23 Years

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In 1917 and again in 1924, Crowe and his associates reported observations upon patients from whom tuberculous tonsils or adenoids had been removed (1, 2). The 1917 report presented a study of 46 patients during the operative period and during the first several years subsequent to operation. The second report dealt chiefly with 50 patients most of whom had been under observation for from 5 to 10 years following operation. These studies led to the conclusion that tuberculosis of the tonsils or adenoids, though frequently accompanied by tuberculosis of the cervical lymph nodes, is rarely followed by active tuberculosis elsewhere in the body. In 1917 most of this group of patients were children; in 1924 many of them were adolescent; at present all of them are adults. It was believed that, if a sufficient number of them could be located and reexamined, valuable information might be obtained concerning the outlook for persons who in early life have been known to have had tuberculosis of the pharyngeal and cervical lymphoid tissues.

At the outset attention should be directed to the fact that the patients to be considered did not have clinically recognized tuberculosis of the tonsils and adenoids. The pharyngeal tuberculosis was probably an incident in a more wide-spread infection. This opinion is based not only upon our own observations, but upon the investigations of others (6), which indicate that isolated, primary tuberculosis of the tonsils and adenoids is rarely, if ever, encountered. Such being the case, the removal of the few scattered tubercles, accomplished by tonsillectomy and adenoidectomy, could scarcely have had any direct

effect upon the tuberculous lesions situated elsewhere in the body. Although it is believed that the excision of the infected and hypertrophied lymphoid tissues resulted in an improvement in the patient's general health, this phase of the problem is beyond the scope of the present investigation. Here, we are interested in the tonsillectomy and adenoidectomy only in so far as they served to establish the diagnosis of tuberculosis.

METHODS

An attempt was made to locate 79 individuals who had had tuberculous tonsils or adenoids removed between 1912 and 1920.[1] Included in this list were all of the 46 patients whose tabulated case records appeared in the 1917 report, and, 33 other patients whose operations were performed between 1915 and 1920.[1] Out of the 79 cases investigated we were able to obtain information concerning 61. Of these, 7 had died, 37 returned to the hospital for examination,[2] and 17 gave information either by letter or by interview with a social worker. The time interval between operation and final note was 15 to 23 years except in 3 cases in which the intervals were 11, 11 and 14 years, respectively.[1,2]

Each patient who returned to the hospital was carefully questioned concerning his health after operation. Specific questions were always asked concerning general health, occupation, hospital or sanatorium care, febrile illnesses, headaches, colds, sore throats, nasal obstruction, pneumonia, pleurisy, cough, expectoration, chest pain, glandular swellings in the neck or elsewhere, menstrual function in women, and body weight. A general physical examination was made, in which special emphasis was placed upon the examination of the lungs. A complete nose and throat examination was also made. The height, weight, temperature, pulse rate, respiratory rate and blood pressure were recorded.[3] An x-ray picture of the chest was taken.

Detailed information concerning operative procedures, histological studies of excised tissues, and postoperative treatment will be found in the earlier reports (1, 2).

RESULTS

Some of the significant data concerning the 61 patients who were located will be found in Tables I and II. In order to facilitate refer-

[1] In the case of patient No. 74 the operation was performed in 1924.

[2] All patients except two were examined between January and June, 1935. Patients No. 41 and No. 73 were examined in 1929, respectively, 14 and 11 years after operation. Patient No. 41 could not be located in 1935. Patient No. 73 is known to be alive and well.

[3] These measurements revealed no significant deviation from average normal values except as noted in Tables I and II.

TABLE I

Group I. Patients under 14 years of age at time of operation

Key to Tables I and II. Column headed *Sex, Age, Race* gives age at time of this study. Figures enclosed in parentheses indicate age at time of death.

Family History Tbc. refers to history obtained at time of operation. The plus (+) signs indicate the number of members of immediate family having tuberculosis. A question mark (?) signifies lack of information.

In columns headed *Cerv. Lymph Nodes* one plus (+) indicates palpable and slightly enlarged cervical nodes, two pluses (++), distinctly enlarged nodes. "Tbc. clinical" indicates a pre-operative clinical diagnosis of tuberculous cervical adenitis, "Tbc. surgical," tuberculous adenitis confirmed by surgical removal of tissue.

X-ray diagnoses enclosed in quotation marks could not be confirmed owing to the loss of the original films.

The following *abbreviations* are used: *A:* adenoids, *G:* cervical lymph nodes, *L:* lungs, *P:* pleura, *S:* spine, *T:* tonsils. In the last column *L & W* means "living and well."

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	Genera	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age Date	Removed		Physical exam.	X-ray					Physical examination	X-ray		
1 H. W. S29528	F 27 W	?	4 1912	T & A	Tbc. Clinical	Clear	Not done	T G	Good health (report)						L & W
2 F. C. S33258	M 27 W	0	5 1913	T & A	Tbc. Clinical	Clear	Not done	T G	Good health (report)						L & W
3 R. S. S33330	M 25 W	0	4 1913	T & A	++	Clear	Not done	A	Good health 1923: X-ray lungs clear	0	0	Clear	Clear	0	L & W
5 N. N. S36033	M 24 C	0	4 1915	T & A	++	Questionable dullness both apices	Not done	T	Good health 1915–1916: X-rays: "enlarged mediastinal glands" (?)	0	+	Clear	Clear	0	L & W
6 E. M. S29388	M 30 W	0	8 1912	T & A	++	Questionable dullness both apices	Not done	A	Good health 1922: X-ray: lungs clear	0	+	Clear	Clear	0	L & W

7 R. B. S30491	M 33 W	0	10 1912	T & A	0	Clear	Not done	T	Good health (report)						L & W
8 R. H. S31504	M 30 C	?	8 1913	T & A	Tbc. Clinical	Clear	Not done	T G	Good health	0	0	Clear	Clear	0	L & W
9 C. M. S32187	M 30 W	0	9 1913	T & A	++	Clear	Not done	A	Good health 1915: X-ray: "clouding lt. apex; mediastinitis; some infiltration just below rt. clavicle" (?)	0	0	Clear	Small calc. nodule 1st. lt. interspace. Calc. deposits lt. lung root	0	L & W
10 H. P. S33070	M 34 W	0	12 1913	T & A	++	Clear	Not done	T	Good health	0	+	Refused	Refused		L & W
11 A. C. S33385	M 32 C	0	10 1913	T & A	++	Clear	Not done	A	Good health	0	0	Clear	Clear	0	L & W
12 G. R. S35579	F 27 W	0	7 1914	T & A	++	Clear	Not done	A	Good health	0	0	Clear	Clear	0	L & W
13 E. K. S36818	M 27 W	0	7 1915	T & A	++	Clear	Not done	A	Good health (report)						L & W
14 C. N. S37805	F 26 W	0	7 1915	T	Tbc. Clinical	Clear	Clear	T G	Good health Tbc. glands excised 1916 1922–1924: X-rays: "lungs clear"	G	0	Clear	Clear	0	L & W
15 A. S. S37093	M (9) C	?	8 1915	T & A	Tbc. Clinical	Clear	Not done	T G	Died tuberculous meningitis 7 mos. after operation	+				+	Died 1915

TABLE I—*Continued*

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	General	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age Date	Removed		Physical exam.	X-ray					Physical examination	X-ray		
16 E. R. S38151	F 30 W	+ +	10 1915	T & A	++	Clear	Calc. deposits lung roots	T	Good health (report)						L & W
17 O. F. S38597	F 30 C	?	10 1915	T & A	+	Clear	Not done	T	Good health (report) 1923: X-ray: lungs clear						L & W
20 F. N. S31486	M 31 W	?	9 1913	T & A	Tbc. Surgical	Clear	Not done	T G	Good health (report)						L & W
21 M. D. S33468	F 32 W	+	11 1913	T	Tbc. Surgical	Clear	Not done	T G	Good health 1913–1914: X-rays: Lungs clear 1915: Tbc. glands drained	G	+	Clear	Clear	0	L & W
23 M. F. S35652	F 34 W	0	13 1914	T & A	Tbc. Surgical	Clear	Not done	T G	Good health (report)						L & W
24 I. G. S37485	M 33 W	0	12 1915	T & A	++	Clear	Not done	A	1923: X-ray: Small calcified nodule lt. apex. Otherwise clear. Chronic bronchitis for several years	0	0	Emphysema. Scattered sibilant râles	Emphysema. Increased lung markings. Calc. nodule lt. apex	0	L & W

26 E. B. S37521	M 32 C	?	12 1915	T & A	+	Clear	Not done	A	Good health (report)						L & W
28 A. D. S38539	M 32 W	0	13 1915	T & A	++	Clear	Not done	T	Good health	0	+	Clear	Small calc. nodules beneath rt. clavicle	0	L & W
29 C. M. S38651	F 31 W	?	11 1916	T & A	Tbc. Surgical	Clear	Not done	T G	X-ray 8 mos. after operation: "suspicious involvement of rt. apex." (?) Good health (report)						L & W
49 E. M. S38899	F 28 C	0	10 1916	T & A	+	Clear	Not done	T	1922-1923 X-rays: lungs clear Good health	0	0	Clear	Clear	0	L & W
50 D. G. S42114	M 25 W	+	5 1916	A	++	Clear	Not done	A	Tonsillectomy 1 yr. later: No tbc. found Good health	0	+	Clear	Clear	0	L & W
51 E. R. S39360	F 25 W	0	6 1916	T & A	Tbc. Surgical	Clear	Not done	T A	1922: X-ray: lungs clear Good health	0	+	Clear	Clear	0	L & W
53 M. W. S36474	F 25 C	0	6 1916	T & A	++	Pleurisy lt. base	"Pleurisy lt. base"	A P	1922: X-ray: numerous calc. nodules periphery & roots both lungs Good health	0	0	Clear	Same calcium deposits as seen in 1922	0	L & W
54 D. B. S39973	F 21 W	+	2 1916	T & A	+	Clear	Not done	A	1918: "double pneumonia" 1922: X-ray: calc. deposits lung roots 1925: active mediastinal tbc. Sanatorium 14 mos.	L	+	Clear	Same calcium deposits as seen in 1922	0	L & W

TABLE I—*Continued*

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	General	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age Date	Removed		Physical exam.	X-ray					Physical examination	X-ray		
55 H. K. S41139	F 28 W	0	9 1916	T & A	++	No note	"Clear"	A	1922: X-ray: calc. nodule periphery lt. lung Calc. deposits both roots 1930: Second T & A	0	0	Clear	Same calcium deposits as seen in 1922	0	L & W
56 V. G. S40650	M 25 W	0	6 1916	T & A	+	Clear	Not done	A	1913: Tbc. hip 1922: X-ray: calc. deposits lung roots 1925: X-ray: calc. deposits lung roots	0	0	Clear	Same calcium deposits as seen in 1922	0	L & W
58 B. W. S40343	M 21 C	+	2 1916	T & A	++	Questionable dullness rt. apex	Clear	A	1923: Well. X-ray: "enlarged mediastinal glands" Good health (report)						L & W
59 J. D. S40946	M 31 W	++ ++	12 1916	T & A	++	Clear	Not done	A	1916: (6 mos. after operation) Well. X-ray: "clouding rt. upper; calc. glands rt. lung root" Good health (report)						L & W

60 A. S. S38847	M 29 W	0	10 1916	T & A	Tbc. Clinical	Questionable dullness lt. apex	"Mediastinitis"	T G	X-ray (8 mos. after operation) "mediastinitis; clouding both apices" 1918–1919: lungs clear 1928: last seen in hospital: well Good health (report)						L & W
61 M. W. F44081	F 21 W	+	3 1916	A	++	Clear	Calc. nodule periphery lt. lung	A	1922: X-ray: lungs clear. Calc. nodule as noted in 1916	0	0	Clear	Calc. deposits lt. lung root. Calc. nodule as noted in 1916	0	L & W
62 H. W. S41703	F 31 C	+	12 1917	T & A	++	Clear	Not done	A	X-ray 5 mos. after operation: "large mediastinal glands" 1922: X-ray: lungs clear 1931: Acute pneumonia	0	+	Questionable dullness both apices	Clear	0	L & W
64 J. H. S42363	F 24 C	0	6 1917	T & A	+	Clear	"Spotty infiltration beneath both clavicles"	A	Good health 1918: X-ray: "spotty infiltration rt. upper" 1922: X-ray: "moderate fibrosis non-tuberculous"	0	+	Clear	Clear	0	L & W
65 R. B.	F 23 W	0	6 1917	T & A	+	Clear	Clear	A	Good health X-ray 6 mos. after operation: "lungs clear" 1934: Acute pneumonia	0	0	Clear	Clear	0	L & W
66 J. H. S43417	M (11) C	0	4 1917	T & A	Tbc. Surgical	Clear	Not done	T A G	1917: Rheumatic heart disease 1920: Acute nephritis (lungs clear) 1924: died. Autopsy: Rheumatic heart disease. No tbc.	0					Died 1924

TABLE I—*Concluded*

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	General	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age Date	Removed		Physical exam.	X-ray					Physical examination	X-ray		
67 A. J. S44591	F 27 C	0	9 1918	T & A	+	Slight dullness rt. apex	Not done	A	1923: No symptoms. Slight dullness rt. apex. X-ray: fibroid infiltration both uppers Good health	L(?)	+	Dullness and bronchial breath sounds rt. upper. No râles	Fibroid infiltration, rt. upper	0	L & W
68 F. F. HLH 14403	F 20 W	+	3 1918	A	++	Clear	Calc. deposits rt. lung root (1917)	A	1922: X-ray: same; lungs clear 1921: tonsillectomy elsewhere Good health	0	+	Clear	Calc. deposits rt. lung root. Same as 1917 & 1922	0	L & W
71 J. K. S50425	F 19 W	0	4 1920	T & A	Tbc. Surgical	Clear	Not done	T G	Cervical glands incised after T & A 1922: X-ray: lungs clear Good health	0	0	Clear	Clear	0	L & W
72 M. P. S51082	F (13) W	+	11 1920	T & A	++	Clear	Calc. glands lt. lung root	T	Congenital syphilis involving central nervous system Epilepsy Died 1922 during convulsions	0					Died 1922

74 A. U. S64144	F 18 W	0	7 1924	T & A	+	Clear	"Large mediastinal glands"	T	1924: Chorea Good health	0	0	Clear	Calc. deposits rt. lung root	0	L & W
77 M. C. S47147	F 21 W	0	4 1918	T & A	Tbc. Surgical	Clear	Clear	T G	Good health (report)						L & W
78 B. B. S39651	M 22 W	+	3 1916	T & A	Tbc. Surgical	Clear	Not done	A G	1922: X-ray: "enlarged mediastinal glands" Good health	0	0	Clear	Calc. deposits both lung roots	0	L & W

TABLE II

Group II. Patients 14 Years of Age or Older at Time of Operation

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	General	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age date	Removal		Physical exam.	X-ray					Physical exam.	X-ray		
19 J. B. S31047	F 36 W	0	14 1912	T	Tbc. Surgical	Clear	Not done	T G	Good health	L (?)	0	Harsh breath sounds, rt. upper. No râles	Several small fibrotic shadows below rt. clavicle and above lt. clavicle	0	L & W
22 M. H. S35638	F 34 C	0	14 1914	T & A	Tbc. Clinical	Clear	Not done	T G	1918: Pleurisy 1923: Polyarthritis 1924: Tubo-ovarian abscess 1935: Hypertension Obese	P	0	Clear	Small fibrotic shadow below rt. clavicle. Calc. deposits lt. lung root	0	L & W
25 M. W. S37485	F 34 W	0	15 1915	T & A	Tbc. Surgical	Clear	Not done	A G	1916: X-ray: enlarged mediastinal glands 1928: Pleurisy Otherwise excellent health	P	+	Dullness lt. base	Thickened pleura both bases, more marked on lt.	0	L & W
27 T. R. S38358	M 34 C	?	14 1915	T & A	+	Clear	Not done	A	Good health (Report)						L & W
30 J. W. S30979	M (24) W	?	19 1912	T & A	++	Dullness lt. apex with râles after cough	Not done	A L	(Hemoptysis prior to operation). Died 1917 of pulmonary tuberculosis	L					Died 1917

37 M. G. S34595	F ? C	0	22 1914	T & A	Tbc. Surgical	Clear	Not done	T A G	1915: Pleurisy 1916: Recurrence of tbc. cervical adenitis Died (date?) of tbc. (?)	P G L(?)					Died (Date ?)
39 N. S. S35504	F 42 W	0	23 1914	T	Tbc. Surgical	Clear	Not done	T G	1915: X-ray: "large mediastinal glands" 1925: Pleurisy Otherwise good health	P	0	Clear	Clear	0	L & W
41 F. W. S38659	F 33 C	0	19 1915	T & A	Tbc. Surgical	Dullness both apices Râles rt. apex	Not done	T A G L	(Latent syphilis at time of operation) 6 mos. after operation: Râles both apices Anti-syphilitic therapy until 1929 Complete examination 1929: well	L	0 1929	Clear 1929	Calcium deposits both lung roots 1929	0	L & W 1929
42 A. H. S38740	F (27) W	++ ++	22 1916	T & A	Tbc. Surgical	Dullness, bronchial breathing rt. apex	Not done	A G L?	8 mos. after operation: dullness and râles lt. apex 1920: Sanatorium, pulmonary tbc. 1920: died, pulmonary tbc.	L					Died 1920
44 J. M. S34742	F 47 W	+	27 1914	T	0	Clear	Not done	T	6 mos. after operation: râles rt. apex 1916: tbc. cervical glands excised 1919: Pleurisy 1920: Pneumonia 1922: X-ray: lungs clear	G P	0	Clear	Calc. nodule 4th left interspace. Same nodule seen in 1922 X-ray	0	L & W

TABLE II—*Concluded*

CASE NO. INITIALS HOSP. NO.	SEX AGE RACE	FAMILY HISTORY TBC.	ORIGINAL STATUS						INTERVAL		PRESENT STATUS				
			Operation		Cerv. lymph nodes	Lungs		Tbc. of	General	Tbc. of	Cerv. lymph nodes	Lungs		Tbc. of	Result
			Age date	Removal		Physical exam.	X-ray					Physical exam.	X-ray		
45 L. R. S35203	M (44) W	0	31 1914	T & A	0	Clear	Not done	T	1924: Hypertension; nephritis 1927: died, chronic nephritis	0					Died 1927
46 O. S. S35291	M 55 W	0	34 1914	T & A	+	Clear	Not done	T	1915: Pulmonary tuberculosis 1916: Tbc. sanatorium 1935: Still has "lung trouble"	L					Living Tbc.
47 H. C. S36205	F 38 W	0	18 1915	T & A	+	Clear	Not done	T	Rheumatic heart disease at time of operation. Good health (report)						L & W
52 M. W. S39377	F 38 C	0	19 1916	T & A	+	Clear	Not done	A	1923: X-ray: lungs clear Good health	0	+	Clear	Clear	0	L & W
63 H. H. S42050	M 41 W	0	22 1917	T & A	Tbc. Surgical	Dullness both apices Râles lt. apex	"Clouding both apices"	T A G	1918–1921: Psoas abscesses 1921: Albee graft spine	S L(?)	+	Dullness and râles right upper	Fibroid infiltration both upper lobes greater on rt. Calc. gland rt. root	L?	L & W
73 F. S. F96589	F 31 C	0	14 1918	A	++	No note	Not done	A	1921: Tonsillectomy, no tbc. 1921: X-ray: lungs clear	0	0 1929	Clear 1929	Clear 1929	0	L & W

ence to the 1917 tabulation, cases numbered 1 to 46 bear the numbers which they bore in the original report (1).

Tonsils and *Adenoids*. These were reexamined in 37 patients. In not a single case did the history or physical examination suggest a recurrence of the tuberculosis of the tonsils or adenoids. The local results achieved by tonsillectomy and adenoidectomy seemed not to differ from those observed in non-tuberculous individuals. In only one instance had there been sufficient regrowth of tonsillar tissue to require a second operation; this was performed 14 years after the first. Unfortunately, the tissue was not examined histologically. In 13 cases there had been no regrowth of tonsils or adenoids. In 11 cases there were small tonsillar tags, and in 10 others small lingual tonsils. In one case there was a rather large mass of adenoids.

Cervical Lymph Nodes. In 44 of the 61 tabulated cases the cervical lymph nodes were considerably enlarged at the time of the original operation. The character of the enlargement was such as to lead to a diagnosis of tuberculous adenitis in 23 cases; in 16 of these the diagnosis was confirmed by histological study of excised tissue. In 4 additional cases tuberculous cervical adenitis developed or progressed subsequent to tonsillectomy, and the involved lymph nodes had to be treated surgically. In each of these instances the operation was performed within two years after the tonsillectomy. No patient gave a history of tuberculous cervical adenitis developing more than two years after tonsillectomy. At the present time in none of the 37 patients examined is there any striking enlargement of the cervical lymph nodes; in 15 there are palpable nodes, which in some cases are quite hard. Roentgenograms of the neck demonstrate that these small, hard lymph nodes contain deposits of calcium.

Intrathoracic Tuberculosis. The incidence of active intrathoracic tuberculosis developing or progressing subsequent to the removal of tuberculous tonsils or adenoids appeared to be higher among those patients whose operations had been performed during adolescence or adult life. For this reason the patients have been divided into two groups. Those included in Group I (Table I) were operated upon before the age of 14 years, (45 patients). Group II (Table II) comprises those patients who were operated upon at 14 years of age or later (16 patients).

(A) GROUP I. Of the 45 patients in this group, all are living except three. None of the deaths was due to intrathoracic tuberculosis:

Patient No. 15 died seven months after tonsillectomy of tuberculous meningitis.

Patient No. 66 died seven years after operation of rheumatic heart disease. At autopsy there was no evidence of tuberculosis.

Patient No. 72 had congenital syphilis involving the central nervous system. Because of mental retardation and repeated epileptiform seizures she was sent to an institution for mental diseases, where she died two years after tonsillectomy in *status epilepticus*. There was no autopsy but during a stay of sixteen months in the institution there had been no clinical evidences of tuberculosis.

Of Group I 42 patients are still living. Of these 14 did not return for examination but reported that they were in good health. Of the 28 who were examined at the hospital all are leading normal, active lives and consider themselves to be in good health.

In none of the patients in Group I were there clinical signs of active intrathoracic tuberculosis at the time of tonsillectomy, with the possible exception of one child (Patient No. 53) who had a fibrinous pleurisy. Patient No. 67 showed "slight but definite" dullness to percussion over the apex of the right lung and there was questionable apical dullness in several other cases. Otherwise the lungs were entirely clear on physical examination; in no case were râles heard. Except in a few cases the original x-rays,[4] taken either at the time of operation or within a few years thereafter, showed the lungs either to be clear or to contain calcium deposits of the type seen after the healing of childhood pulmonary or mediastinal tuberculosis. The exceptions were a few subjects with enlarged but not calcified "mediastinal glands," several cases (original x-rays lost) said to have shown

[4] *X-ray studies* of the lungs prior to operation were made in only a few cases and even in these the reported interpretations of the films are not to be accepted without a certain amount of scepticism. Such diagnoses as "mediastinitis," "parenchymal clouding" and "spotty infiltration" depended upon criteria which would not be accepted as indicative of tuberculosis in present-day interpretations of roentgenograms. In fact, some of the original films which led to such diagnoses, when reexamined appeared to be quite normal. For this reason we have not put much value upon x-ray reports where the original films have not been available for examination. In cases in which the films have been lost the original diagnoses have been entered, in quotation marks, in Tables I and II.

"clouding" in the periphery of the lungs, one case of apical "infiltration," and one case of pleurisy.

Since the time of operation only one patient has developed symptoms or signs indicating active intrathoracic tuberculosis:

Patient No. 54, whose tonsils were removed at the age of 2 years, was repeatedly exposed to infection from an aunt who had pulmonary tuberculosis. Two years after operation she had what was called "double pneumonia." Four years later an x-ray of the chest showed calcium deposits in the right lung root. Nine years after operation, at 11 years of age, she developed a cough and was sent to the State Tuberculosis Sanatorium where she remained for 14 months. A report from the sanatorium includes the following information: "Her sputum was negative, but the x-ray showed heavy roots with calcification in both hili, extending up into the apices. Her temperature range for the first three months was 99°; normal after that. She gained 10 lbs. while here." The diagnosis was "childhood type of tuberculosis." Since leaving the sanatorium she has felt quite well and has been working regularly. She is now 21 years old; the lungs are clear except for calcium deposits in both roots.

At the present time none of the 28 patients in Group I who returned to see us in the hospital shows any signs of active pulmonary tuberculosis. One patient (no. 67) has significant impairment of the percussion note at one apex but there are no râles and the x-ray picture reveals only a small fibroid apical lesion. This lesion was noted in an x-ray picture taken in 1923 and it was probably present at the time of operation in 1918 when there is said to have been dullness at the right apex. Of the 27 x-ray films (one patient refused to submit to x-ray examination) 11 show calcium deposits in the periphery of the lungs, in the lung roots or in both of these locations. In 7 of these cases x-ray pictures taken at the time of operation or within several years after operation were available for comparison with the recent films. In all instances the calcium deposits were visible in the original films.

In *summarizing* the experience with the 45 patients in Group I, it may be said that the incidence of serious tuberculous sequelae was very low. Except for one patient who died of tuberculous meningitis 7 months after operation there have been no deaths from tuberculosis. As far as can be determined no patient in the group has developed an adult type of pulmonary tuberculosis since the removal of the tuberculous tonsils or adenoids. One child who was exposed to tuberculosis in the family developed active mediastinal tuberculosis 9 years after

operation, but this responded promptly to sanatorium treatment. Another patient shows, on x-ray examination, slight fibrosis at one apex, but this was probably present at the time of operation. More than a third of the patients subjected to x-ray examination show in their lungs calcium deposits, indicating the occurrence of a childhood type of tuberculosis. In at least some of these cases, possibly all of them, the deposits had been laid down prior to operation.

(B) GROUP II. Of the 16 patients in this group 4 have died. One death was definitely not due to tuberculosis:

Patient No. 45, following operation, was submitted to repeated sputum and chest examinations which were always negative for tuberculosis. Ten years later he developed hypertension and chronic nephritis which led to his death 13 years after tonsillectomy. There was no autopsy.

The other three patients who died all probably had intrathoracic tuberculosis before death:

Patient No. 30 had had a small haemoptysis in 1912, 3 weeks before coming to the hospital, but at the time of operation his lungs were thought to be clear (no x-ray). Five days later, however, dullness and râles were noted at the right apex. After three years had elapsed the patient reported that he had a chronic cough and had been in poor health ever since his operation. A letter from his father states that he "died of tuberculosis" 4½ years after operation.

Patient No. 37, on physical examination at the time of tonsillectomy, was found to have clear lungs (no x-ray). Nineteen months after operation she had an attack of pleurisy. When she returned to the hospital 2 months later, there were enlarged and obviously tuberculous lymph nodes in the opposite side of the neck from those excised at the time of tonsillectomy. There is no record of any examination of the chest at this time. She did not return again to the hospital and it is only known that she died within the next several years.

Patient No. 42 had undergone two operations for tuberculous cervical adenitis before coming to the hospital in 1916. At the time of tonsillectomy dullness and bronchovesicular breath sounds were noted at the right apex. Eight months later dullness and fine râles were noted at the left apex. Four years after operation she was admitted to the State Tuberculosis Sanatorium. A report from the Sanatorium states that at that time "she was running a very acute course with temperature up to 103° daily, and frequent hemorrhages." The sputum was positive for tubercle bacilli and an x-ray of the chest showed spotty infiltration of both upper lobes and consolidation of the left lower. She remained in the sanatorium 3½ months and died shortly after returning to her home.

Of the patients in Group II eleven are known to be alive, and a twelfth, although she cannot be located at the present time, was in good health when last thoroughly examined in 1929. Three patients who were located failed to return for examination: two reported that they were in good health, the third (Patient no. 42) reported that he "still has lung trouble." In the last case the patient's lungs, at the time of operation had been considered normal (no x-ray), but 3 months later active apical pulmonary tuberculosis was discovered and the patient was sent to a sanatorium. Since that time he has had "weak lungs and a cough" and has been living in the mountains.

Of the nine patients who returned to the hospital for examination[5] seven give a history or show physical or x-ray signs suggesting intrathoracic tuberculosis:

Patient No. 19 gives no history of pulmonary symptoms but has physical signs and x-ray evidences of apical fibrosis.

Patient No. 22 had pleurisy 4 years after operation and the x-ray now shows fibrotic shadows in the right upper lobe.

Patient No. 25 had pleurisy 13 years after operation and now has physical signs and x-ray evidences of thickened pleura at the lung bases.

Patient No. 39, 11 years after operation, developed a cough, pain in the chest and fever which were said to be due to pleurisy. During a month's illness she lost 25 lbs. in weight. Her convalescence from this illness was uneventful and the weight was gradually regained. At present there are no evidences of intrathoracic tuberculosis.

Patient No. 41, 6 months after operation, developed râles at both apices. These gradually disappeared. When examined in 1929, the lungs were clear except for calcium deposits in the roots.

Patient No. 44 developed râles at the right apex 6 months after operation. A year later tuberculous cervical adenitis appeared and the infected glands were excised. Five years after operation there was an attack of pleurisy and a year later an attack of "pneumonia." An x-ray, made 8 years after operation, showed only a small calcified nodule in the periphery of the left mid-lung field. The lungs are now clear except for the presence of this same nodule.

Patient No. 63, at the time of operation, had dullness at both apices, râles at the left apex, and the x-ray showed "clouding of both apices." A year later signs of bilateral psoas abscesses began to develop which eventually required surgical treatment. A spinal bone-grafting was done 4 years after tonsillectomy.

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[5] Two of these examinations were made in 1929.

Since that time the patient has felt well and has been working regularly. Although there have been no symptoms of pulmonary disease, there are now x-ray evidences of fibroid infiltration in both upper lobes and there are râles at the right apex.

In *summary* the experience with the 16 patients in Group II has revealed a high incidence of intrathoracic tuberculosis (11 patients). Progressive pulmonary tuberculosis of the adult type occurred in three patients and possibly in a fourth who died while not under observation. Apical pulmonary fibrosis was discovered in three other patients, one of whom now has moderately extensive fibroid infiltration in both upper lobes. The patient who died while not under observation and four additional patients developed pleurisy subsequent to operation. The available information does not allow one to determine how many of these patients had pulmonary tuberculosis at the time of operation. Some of them certainly did. In all cases in which symptoms of pulmonary tuberculosis developed they made their appearance within a few years, usually within several months of the operation. However, four patients developed pleurisy 4, 5, 11 and 13 years, respectively, after operation. In addition to intrathoracic tuberculosis three patients in Group II developed, subsequent to tonsillectomy, other forms of tuberculosis: two, cervical adenitis and one, bilateral psoas abscesses.

DISCUSSION[6]

Of the 79 patients subjected to investigation, 32 were 14 years of age or older at the time of operation. Only 16 of these patients (Group II) could be traced. This group is so small that it is difficult to reach any conclusion concerning the significance of the pharyngeal tuberculosis. Newhart, Cohen and Van Winkle (4) have reported a high incidence of histological tuberculosis of the tonsils in adults having pulmonary tuberculosis: 48 per cent of 100 patients having active pulmonary tuberculosis; 57 per cent of 71 patients having tubercle bacilli in the sputa. Unfortunately, the pre-operative studies on our patients were too incomplete, and x-ray examinations were too sporadic to allow one to estimate, in retrospect, the incidence of pulmonary tuberculosis at the time of discovery of the tonsillar lesion.

[6] The conclusions reached in the study of these patients have already been presented in abbreviated form (7).

The available evidence indicates that a considerable number of the patients in Group II had pulmonary tuberculosis of the adult type at the time of operation.[7] This, taken in conjunction with the well recognized sharp increase in the tuberculosis mortality rate at the time of puberty, doubtless accounts for the relatively poor prognosis in the group of older patients.

Of the 47 patients in the younger group (Group I) 45 could be traced. The two patients who could not be located were well when last examined 22 months and 15 months, respectively, after operation. Of the 45 patients traced, twenty-two had been operated upon 20 or more years previously, twenty-two, 15 to 19 years previously, and one, 11 years previously. Not a single patient in this group had developed progressive pulmonary tuberculosis after operation.

In Group I, as well as in Group II, the pre-operative studies of the patients were too inadequate to allow one to estimate, in retrospect, the extent of the original tuberculosis. A review of the x-ray studies indicates that in a fair proportion of the patients of Group I the lungs and mediastinal lymph nodes were involved in a childhood type of tuberculosis at approximately the time of the operation. In a much larger proportion of the patients there is clear evidence that the cervical lymph nodes were involved. A pre-operative diagnosis of tuberculous cervical adenitis was made in 17 of the original 47 patients, and it seems almost certain that many of the greatly enlarged lymph nodes that were present in twenty additional patients were the seat of tuberculous infection.

The high incidence of tuberculous cervical adenitis in the pre-adolescent group has interested us particularly, in view of the controversy concerning the prognosis in this form of tuberculosis. The complete absence of progressive pulmonary tuberculosis among these patients is in keeping with the observation, recently re-emphasized by Marfan (3), that individuals whose scrofulous lesions heal prior to the age of 15 years rarely develop progressive tuberculosis in later life.

Attention should be directed to the fact that tuberculosis of the

[7] Of the sixteen patients in this group who could not be located, at least three had intrathoracic tuberculosis at the time of operation: two cases of apical fibroid tuberculosis confirmed by x-ray, and one case of thickened pleura (x-ray) in which the patient gave a history of having had pleurisy with effusion.

tonsils in pre-adolescent children is not always to be looked upon as an indication of a benign form of tuberculosis. Otto (5) in autopsy studies found tubercles in the tonsils in a high percentage of children dying of tuberculosis before the age of 12 years. In these cases the tuberculosis had generally run a fulminating course, and in many of them the tubercles in the tonsils were considered to be part of a generalized miliary tuberculosis.

The foregoing observations have led to the belief that when one discovers tubercles in the tonsils, adenoids or cervical lymph nodes of pre-adolescent children the immediate prognosis depends upon the character of the disease elsewhere in the body. Death may occur from the progress of the existing childhood disease, but if the patient recovers from the latter, he is, according to our experience, not likely to develop adult forms of tuberculosis during the ensuing fifteen to twenty years.

Additional studies of this type will be necessary before one can arrive at conclusions which are statistically reliable.

SUMMARY

1. Seventy-nine patients discovered at operation, between 1912 and 1924, to have tuberculosis of the tonsils or adenoids, were subjected to reinvestigation in 1935.

2. Of forty-seven patients operated upon prior to the age of 14 years, forty-five could be traced. Two had died of non-tuberculous diseases, and one, of tuberculous meningitis several months after operation. All of the forty-two living patients were well, and there was no evidence that any of them had developed pulmonary tuberculosis of the adult type subsequent to operation. The two patients who could not be traced were well when last seen, 15 to 22 months after operation.

3. Of thirty-two patients operated upon when past the age of 13 years, only sixteen could be traced. One had died of non-tuberculous disease. At least two and possibly three had died of pulmonary tuberculosis. Of the twelve patients known to be alive, one has progressive pulmonary tuberculosis, and three others have fibroid apical pulmonary tuberculosis. Two additional patients had at one time had physical signs of apical tuberculosis. Five patients gave a history

of pleurisy. Of the sixteen patients who could not be traced, three had intrathoracic tuberculosis at the time of operation.

4. The available evidence indicates that the relatively poor prognosis in the older group was attributable to the existence, at the time of operation, of pulmonary tuberculosis of the adult type.

5. The prognostic significance of tuberculosis of the tonsils, adenoids and cervical lymph nodes in pre-adolescent children is discussed.

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DIETARY PROTEIN AND THE REGENERATION OF SERUM ALBUMIN

I. Method of Assay and Discussion of Principles

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Among the therapeutic measures which have been proposed for relieving the edema caused by low concentrations of albumin in the serum, none has been commended more highly than the use of diets high in protein. With some patients only is the response satisfactory. In certain instances its failure appears to be explained by the presence of albuminuria, anorexia, vomiting or diarrhea; in other cases such complicating symptoms are either absent or their intensity is so slight that the reason for retarded albumin formation is obscure and one is brought to suspect some defect in the mechanism for synthesizing the protein. The investigations to be described in this and subsequent papers represent an attempt to learn whether better results can be hoped for if consideration is given to the quality of the protein in the diet as well as to its quantity. The experiments have as their goal the classification of the more important food proteins with respect to their ability to promote the synthesis of serum albumin.

LITERATURE

Liu, Chu, Wang and Chung (1) have reported observations on two cases of nutritional edema in which complete studies of the nitrogen balance and plasma proteins were made over a space of three months. The paper is pertinent to the present discussion because of the conclusion: "It seems that 1 gram of animal protein per kilo is much superior to the same amount of vegetable protein *in building up plasma proteins* and reducing edema, and that 2 grams of vegetable protein seem necessary to secure the same effect as produced by 1 gram of animal protein." A study of the data in this report renders it clear that the statement regarding the building up of plasma protein could not have been made on the basis of measurements of plasma protein concentration; the authors merely

showed that the nitrogen balances were about equally positive on a diet which furnished 7 per cent of the calories as animal protein (pork and egg), and on a diet in which 14 per cent of the calories were from vegetable protein. With case I, for example, the serum albumin rose from 2.18 to 2.67 gm. per cent on the 7 per cent vegetable protein diet; during the period of feeding with 7 per cent animal protein the highest albumin level was 2.53 gm. per cent; on the subsequent 14 per cent vegetable protein diet a level of 3.24 gm. per cent was attained. The data for both patients show that even on the best diets serum albumin was formed slowly and with difficulty.

Liu and Chu (2) reported metabolic observations on two patients with the nephrotic type of Bright's disease during periods when the energy content of the diet and the quantity and quality, *i.e.*, whether of animal or of vegetable origin, of the protein component were varied. They were able to determine the conditions which led to the maximum storage of nitrogen in the body but they failed to show that such storage had an appreciable effect in altering the concentration of proteins in the serum.

Keutmann and Bassett (3) made a thorough metabolic study of two patients with chronic Bright's disease whose dietary was supplemented from time to time by the addition of varying amounts of lactalbumin, beef serum protein, liver protein, and egg white. The addition of protein to the diet resulted in an increase both in retention of protein in the body and in excretion of protein in the urine. Although the increased proteinuria could be interpreted as evidence of increased formation of serum protein, it was disconcerting to observe no significant change in the total amount of serum protein or in its concentration.

In three papers Whipple and his associates (4) have described experiments on dogs in which various food proteins are classified with respect to their ability to promote the formation of total plasma protein. During maintenance on a constant basal diet, dogs were subjected to daily plasmapheresis, the volume of the bleedings being regulated so as to maintain a plasma protein concentration in the neighborhood of 4 per cent. After a period of adjustment, the basal plasma protein production was calculated as the grams of plasma protein removed in the bleedings per week. The basal diet was then supplemented by adding the food protein to be assayed. Following such additions it was always necessary, in order to keep the plasma protein level at 4 per cent, to increase the volume of plasmapheresis. The plasma protein removed in excess of the basal production was credited to the added food protein. For each food protein Whipple calculated a "potency ratio" which represented the grams of the food protein required to produce the synthesis of one gram of serum protein. Most of the foods were classified on the basis of a single assay. A few typical potency ratios are: beef serum, 2.6; gizzard, 5.3; lactalbumin, 5.5; skeletal muscle, 5.7; egg white, 5.9; potato, 6.1; liver, 6.4, 6.5, and 6.8; soy bean, 7.1; casein, 10; salmon, 15.2. The protocols of the experiments contain data regarding the ratio of albumin to globulin in the plasmas; the data allow recalculation of the various food potencies in terms of ability to promote the formation of serum albumin. Since such

recalculated potencies can be compared with the findings in this investigation, there will be occasion to refer to them in subsequent papers.

Melnick, Cowgill, and Burack (5) studied on two dogs the relative effects of beef serum protein, lactalbumin and casein in promoting the formation of total serum protein. Like Whipple, they made use of plasmapheresis to maintain a stimulus for serum protein formation, but they also tried to control a number of variables which they thought had been ignored in the earlier work. They calculated the "potency ratio" of a food as (a) the amount of serum protein removed per week by the bleedings, above that regenerated when the animal was eating a basal protein-free diet, divided by (b) the amount of dietary protein in excess of that required for nitrogen equilibrium. This potency ratio bore an inverse relationship to the one formulated by Whipple and possessed the advantage that the more potent the protein the higher the potency ratio. The ratios obtained in this work were: serum protein, 0.53 and 0.64; lactalbumin, 0.38 and 0.34; casein, 0.45 and 0.42. These investigators did not determine the ratio of albumin to globulin in the sera of the dogs and for this reason their results cannot be compared with those to be reported by us.

In summarizing the results in the literature, it is important to note the items wherein disagreement exists. Observations on dogs by two groups of investigators, both of whom utilized the method of plasmapheresis, have shown that the quality of dietary protein exerts an effect on the rate of formation of serum protein. Observations on humans with chronic Bright's disease and with nutritional edema have not shown that one type of dietary protein is more effective than another in raising the concentration of serum protein in the circulation. Although one might infer that the discrepancy is merely the result of species specificity, the circumstances do not warrant such a conclusion. The maintenance of hypoproteinemia in dogs by repeated plasmapheresis may evoke a stimulus to plasma protein regeneration which is different from that required to raise a lowered concentration toward normal, and it is not unlikely that conditions surrounding the development of naturally-occurring types of hypoproteinemia may greatly complicate the process of regeneration. We have, therefore, hoped that a new plan of investigation might result either in disclosing new facts concerning the quality of protein best suited for plasma protein regeneration or in explaining why the hypoproteinemic human often fails to respond when good protein is fed.

METHOD OF ASSAY

The method which is being used to assay the food proteins is simple. A healthy dog is selected and placed under daily observation for 6 days

on a standard diet of adequate protein content. This standard diet is a mixture of 900 grams of our basal low protein diet (6) with 90 grams of casein. The concentration of albumin in the serum is then measured; the casein is removed from the diet and the period of depletion of the serum albumin is begun. After 3 weeks the serum albumin is again determined and the period of regeneration started on a diet composed of a mixture of the basal diet and the food protein to be tested. The mixture is fed at an energy level of 80 calories per kilo and at a protein level of 2.5 grams per kilo (12.6 per cent of calories). After one week on this diet the final measurement of serum albumin concentration is made.

The initial albumin analysis is a control determination only; at present the result has no direct part in evaluating the assay. The difference between the third and the second analyses gives the rise in serum albumin concentration during the week when the test protein was fed. This figure might be used as a measure of the potency of the food. However, there is some advantage (to be pointed out later) in making allowance for the fall in albumin concentration which would have occurred during the fourth experimental week if the test protein had not been fed. The prevention of this decline should also be credited to the food. In 23 control experiments in which the basal low protein diet was actually fed longer than 3 weeks, the average decline in albumin during the fourth week was 0.15 gm. per cent. Accordingly the "assay value" of a food is calculated as the rise in albumin concentration plus 0.15. When assay values are determined for the same food on a number of different animals, the range of biological variation among the results will be fairly wide. It follows that a series of assays must be made before an average value, to be called "the potency value," can safely be assigned to any one protein.

TECHNICAL METHODS

Animals. The dogs used in these experiments were of mongrel breeds; their average weight was 22.6 kilos. They were selected on the basis of good general health and willingness to consume the basal ration. The use of excessively lean or fat animals was avoided. In many instances a single animal has served for the assay of a number of different foods; in such cases an interval of at least 5 weeks has always elapsed between experiments. It has concerned us somewhat as to whether a dog subjected to repeated periods of plasma protein depletion might react differently from the fresh animal. For the most part there has been no

obvious evidence of a difference and the advantage of being able to compare the assays of two foods on the same test animal has seemed to outweigh the theoretical disadvantage.

Diets. The composition and preparation of the basal low protein diet has been described previously (6). One gram of the basal diet contains 0.00854 gm. protein (from carrots and rice) and provides 1.342 calories. The test food proteins were analyzed for nitrogen by the Kjeldahl method and their protein content was calculated as 6.25 times the nitrogen. Analyses for fat and carbohydrate were not made; the values used in calculating the caloric content were taken from the tables of Atwater and Bryant (7). The regeneration diets were mixtures of the basal diet and the test foods, so calculated that each animal received 80 calories and 2.5 gm. of protein per kilo of weight. If x = the grams of basal diet and y = the grams of test food to be fed per kilo and if k_1 = the calories and k_2 = the grams of protein in one gram of the test food, then

$$1.342x + k_1y = 80 \tag{1}$$

$$0.00854x + k_2y = 2.5 \tag{2}$$

The solution of these equations is facilitated by combining equations 1 and 2 so as to eliminate x.

$$y = \frac{312.8}{157.1\,k_2 - k_1} \tag{3}$$

From equation 3, y is obtained directly by substituting the appropriate values of k_1 and k_2; x is obtained by substituting the value of y in equation 1.

It will be observed that maintenance of the total protein at a constant level in the regeneration diet cannot be accomplished without some variation in the relative amounts of protein furnished by the basal diet and the test food. In practice, however, the range of variation is small. For example, the relative amount of test food protein in the total protein of the diet is: for a test food containing only protein, 81.8 per cent; for beef liver, 82.8 per cent; for lean beef chuck, 83.8 per cent.

Methods of measurement. For the determination of the plasma proteins 20 cc. of blood are withdrawn from the femoral artery, the large sample insuring an adequate reserve for checking questionable analytical results. The blood is chilled rapidly and centrifuged within half an hour; handling the sample in this way has been found to minimize hemolysis. Fractionation of the serum protein is done by Howe's method. All nitrogen measurements are made by micro-Kjeldahl technic, the ammonia being distilled in steam into N/70 acid and titrated back with N/70 alkali. Total protein and non-protein nitrogen determinations are run in duplicate. Except for an early series of analyses, measurements of albumin have been made in quadruplicate, duplicate analyses being set up on consecutive days so as to eliminate the possibility of parallel errors in manipulating a single series of flasks. More determinations have been run when good agreement among the replicates was lacking. Statistical analysis of 244

consecutive albumin analyses has revealed a standard error of measurement (SE_M) of 0.042 gm. per cent for a single determination. Recorded results which represent the average of four determinations have a SE_M of 0.021 gm. per cent. The assay value of a test food protein, which depends on the difference between two analyses, has a SE_M of 0.030 gm. per cent. Although in any long series of analyses statistical considerations lead one to expect a few errors as great as three times the standard error, it is unlikely that such extreme variations are represented in the recorded values, since it has been a practice to run more analyses when unusual divergence was evident among replicate results.

Plasma volume was measured by the dye method (8). Comments on technic in applying this method have been given in another place (9).

EXPERIMENTAL RESULTS

The findings to be reported in this paper concern features which have been relevant to establishing the method of assay in its present form. A number of separate but interrelated items will have to be discussed. To facilitate reference the items will be considered in a series of numbered sections.

Section 1. The potency value of casein for the formation of serum albumin. It is not our intention in this introductory paper to report the potency values obtained with various foods. Such values can be located more conveniently in brief subsequent reports. For purposes of discussion, however, it is desirable that the reader be familiar with the type of data secured in the assays. Accordingly, the results obtained with casein will be presented at once.

The casein used was a commercial brand which on analysis showed 13.1 gm. nitrogen per 100 gm. By use of the factor 6.25 a protein content of 81.9 per cent was estimated. The purity of the product was not so low as this figure would seem to indicate. The factor which relates nitrogen to protein is higher than 6.25 for casein. However, because of difficulties which would attend the analysis of many foodstuffs for protein rather than for nitrogen, we have elected to regard as protein a value which bears a constant relation to nitrogen. During the regeneration period each dog received per kilo: 2.496 gm. casein and 53.37 gm. basal diet. The casein was mixed with the basal diet at the time of feeding.

Assays have been performed with twelve dogs. The results appear in Table I. The potency value of casein with its probable error (P.E.) is 0.388 ± 0.027. Nine of the twelve assay values are within ± 0.1 of

the potency value; the other three results cover a wide range, from 0.07 to 0.58.

Section 2. The effect of diet on the formation of serum globulin. There can be no doubt that under certain circumstances globulin formation can be influenced by diet. In previous contributions it was shown that both humans (10) and dogs (6), when given an adequate diet after a long-continued period of protein deprivation, exhibit an immediate rise in globulin which for a time may be greater than the concomitant rise in albumin. Since increases in albumin concentration are associated with increases in plasma volume (9), the

TABLE I

Assays of casein for albumin formation

DOG	SERUM ALBUMIN PER 100 CC.					ASSAY VALUE
	Initial	Depletion	Regeneration	Loss	Gain	
	grams	*grams*	*grams*	*grams*	*grams*	
B-12	3.81	2.63	2.55	1.18	−.08	0.07
5-7	3.61	2.76	2.79	0.85	0.03	0.18
5-31	4.07	3.04	3.25	1.03	0.21	0.36
7-27	3.40	2.27	2.51	1.13	0.24	0.39
6-35	2.89	1.90	2.14	0.99	0.24	0.39
8-04	3.38	2.52	2.77	0.86	0.25	0.40
9-51	3.46	2.73	2.99	0.73	0.26	0.41
7-28	3.16	2.40	2.70	0.76	0.30	0.45
7-47	3.44	2.47	2.78	0.97	0.31	0.46
3-0	3.80	2.91	3.23	0.89	0.32	0.47
9-49	4.60	3.20	3.54	1.40	0.34	0.49
3-36	3.42	2.66	3.09	0.76	0.43	0.58

Potency value: 0.388 ± 0.027 (P.E.).

globulin rise cannot result from plasma concentration and must indicate that diet has stimulated the production of serum globulin. Whipple and his associates (2) have concluded that in general plant proteins favor the production of globulin as opposed to animal proteins, which favor the formation of albumin. It is not easy to appraise their work, since for the most part the plant proteins were fed at a low level and the animal proteins at a much higher level. However, their experiments do show that the output of globulin during plasmapheresis is reduced by fasting.

Notwithstanding these findings it is a matter of common observa-

tion that under usual circumstances the store of serum globulin in the circulation is not greatly affected by the intake of food. After protracted febrile illness with anorexia and loss of weight, it is not uncommon to find the concentration of serum globulin at a level above normal. In a previous report from this laboratory (6), the average findings were reported on twenty-one dogs during maintenance on the basal low-protein diet; average globulin concentration was almost unaffected for as long as 80 days. In a later paper (9), in which attention was called to the decline in plasma volume which accompanies the early weeks of low-protein feeding, it was shown that a small loss of globulin does occur even though concentration is unaffected. However, at the end of 80 days, 90 per cent of the initial quantity was still present. These circumstances make it clear that the internal mechanisms which control the formation of albumin and of globulin operate in different ways and suggest that it is not wise to study the synthesis of serum protein without consideration of its component fractions. In the present attempt to assay various food materials the total protein of serum has varied so widely that dependable conclusions concerning it are impossible.

The findings for serum globulin have been analyzed separately. In 64 assays with various foods the average initial globulin concentration was 3.29 gm. per cent; the level after 3 weeks on the basal diet was 3.37 gm. per cent; the final level after feeding the test food was 3.33 gm. per cent. The average rise in albumin concentration during the final week was 0.45 gm. per cent. Because it might be suggested that the individual fluctuations in globulin depend more on the change in albumin than on the test food, an analysis has been made (Fig. 1) of the relationship between gain in albumin concentration and change in globulin. The changes in globulin are distributed in a random manner and bear no clear relationship to the behavior of the albumin fraction. In Table II the globulin changes are distributed with respect to the test foods which were fed. The table does not reveal that one food has been more or less effective than another in influencing globulin concentration.

Because it might be argued that changes in plasma volume have obscured the significance of the globulin findings, an approximate calculation (see *Section 3*) has been made of the plasma volumes in

each experiment; the calculation has allowed us to express the globulin change in terms of grams of serum globulin per kilo of dog, the weight being taken at the start of the regeneration period. In Fig. 2 these calculated globulin values have been charted against the change in

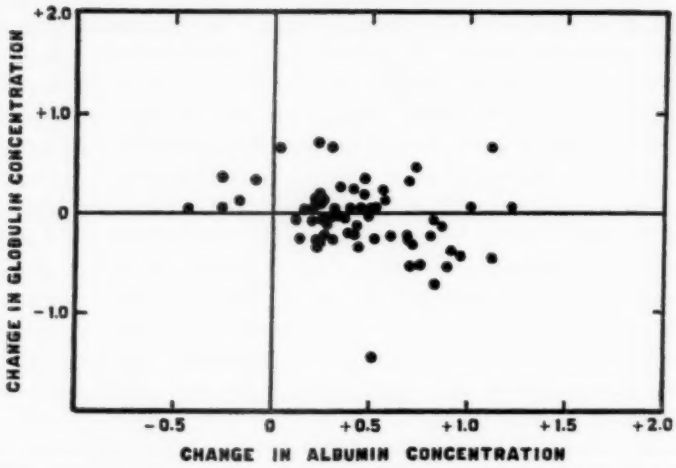

FIG. 1. COMPARISON OF CONCENTRATION CHANGES IN THE SERUM DURING THE FEEDING OF VARIOUS FOOD PROTEINS TO HYPOPROTEINEMIC DOGS

TABLE II

GLOBULIN CONCENTRATION	CASEIN	BEEF CHUCK	BEEF SERUM	MISCELLANEOUS	TOTALS
Increased	10	6	2	12	30
Decreased	7	9	9	9	34
Totals	17	15	11	21	64

Change in concentration of globulin in the serum during the regeneration period. The value of chi-square from this table is 5.87. The value required for significance (P = 0.05) is 7.82. There is no significant difference between any of the test foods.

albumin concentration. Again there is no indication that the albumin change has influenced the behavior of globulin. In contrast to Fig. 1, however, Fig. 2 reveals that most animals do gain in circulating globulin during the period of test protein feeding. It appears then that

loss of globulin during dietary deprivation of protein and subsequent gain with adequate feeding are both demonstrable only when allowance is made for the variations in the volume of the plasma. In Table III the calculated globulin values are arranged with respect to

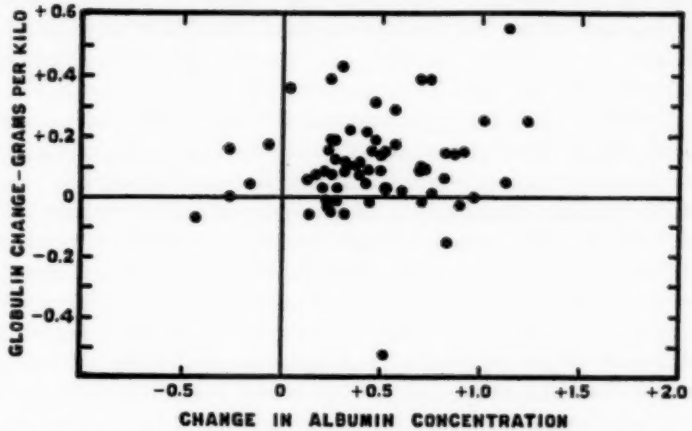

FIG. 2. THE CHANGE IN ALBUMIN CONCENTRATION COMPARED WITH THE CHANGE IN TOTAL CIRCULATING GLOBULIN WHEN VARIOUS FOOD PROTEINS ARE FED TO HYPOPROTEINEMIC DOGS

TABLE III

GLOBULIN INCREASE	CASEIN	BEEF CHUCK	BEEF SERUM	MISCELLANEOUS	TOTALS
Greater than median	11	6	3	12	32
Less than median	6	9	8	9	32
Totals	17	15	11	21	64

Change in globulin during the regeneration period. Globulin expressed as grams per kilo of body weight. The value of chi-square from this table is 4.77. The value required for significance (P = 0.05) is 7.82. There is clearly no evidence of significant difference between any of the test foods.

the several test foods. Again there is no evidence among the foods tested that one is more or less effective than another in promoting the formation of globulin.

The variable and inconsistent character of the foregoing experi-

mental data has made us wonder whether it may not be that the body does not depend greatly on the diet for a source of amino acids from which to synthesize globulin. Such a state of affairs would be entirely reasonable. During infections which are generally attended by anorexia the globulin constitutes an important immune defense mechanism. That the immune mechanisms should not easily be thrown out of order by anorexia would seem to be an important circumstance in our ability to combat infections.

Section 3. Relationship between rise in concentration of albumin and rise in total grams of albumin in the circulation. Estimations of plasma volume were made routinely in the first 40 assays of the study. The estimations allow computation of the total quantity of albumin added to the blood stream after feeding a test food. Although the expression of potency values in terms of effect on concentration has the desirable feature of simplicity, any other mode of expression would be preferable if it led to greater consistency in the results of replicate assays. Failure to obtain such an increase in consistency is the chief reason why estimations of plasma volume are no longer included in the method of assay.

Mathematical analysis of the data from the standpoint of consistency in results proceeded as follows: Ten experiments were excluded either because the test foods failed to produce an appreciable rise in albumin concentration or because the lack of duplicate assays with the same test food prevented any estimate of variability. With the remaining 30 experiments the test foods were so distributed as to permit the calculation of ten independent estimates of variability (standard deviation or sigma). The weighted average of the sigmas was then computed by the method suggested by Treloar (11). These average values of sigma have the same accuracy as if they had been computed from 21 experiments with the same test food. From the sigmas the coefficient of variation (C.V.) was calculated by dividing by the average result in the 30 assays. To determine the effect of consideration of plasma volume on the consistency of assays, the C.V. was calculated for results expressed in three ways: (*a*) for the increment in albumin concentration; (*b*) for the increment in total quantity of albumin per 100 cc. of plasma volume before administering the test food; (*c*) for the increment in total quantity of albumin per kilo of

dog weight. In the order mentioned the values for the C.V. were 31 per cent, 28 per cent, and 30 per cent. No statistical significance can be attached to the small differences. It is of passing interest to note that if calculated rather than determined values for plasma volume are assumed for all experiments and the results calculated by method *b* above, the C.V. is reduced to 23 per cent. This phenomenon, which is being investigated further, may ultimately lead to a correction factor in standardizing the potency values from replicate assays (see *Sections 6 and 9*). At present it appears to depend less on the actual change in plasma volume than on mathematical considerations arising from the fact that the actual values for albumin concentration rather than increment in concentration enter the computation (see *Section 6*).

Some understanding of why the inclusion of plasma volume in the calculation of assay values does not improve significantly the reliability of the results may be obtained if it is recalled that plasma volume appears to be in part a function of albumin concentration (9). Since changes in albumin concentration are usually paralleled by changes in plasma volume, the former measurement by itself includes an estimate of the latter. In the 40 experiments dealt with in this section the relationship (coefficient of correlation) between the increment (gm. per cent) in albumin concentration, Δ_A, and the percentage increase in plasma volume, Δ_v, was 0.75. This circumstance can be utilized, when occasion arises, to estimate the change in quantity of circulating protein when only measurements of concentration have been made (as in *Sections 2 and 6*). For this purpose one begins by assuming an average plasma volume per kilo of 46 cc. at the start of the regeneration period; the percentage rise in plasma volume is then computed from the regression equation:

$$\Delta_v = 14.85\Delta_A + 2.03$$

Section 4. Decline in albumin during the 3-week preparatory period. Limitation to 3 weeks of the preparatory period of low protein feeding has been determined by several circumstances: (a) Neither vitality nor appetite show signs of impairment during this interval. In most animals objective signs of protein deprivation will appear after 6 to 9 weeks. (b) The fall in concentration of albumin in the serum is more rapid during this period than at any time subsequently. To double

the fall obtained in 3 weeks requires about 11 weeks (6). (c) The volume of the plasma invariably declines rapidly during the first 3 weeks but subsequent change is irregular in direction (9). The losses in circulating albumin are therefore greater than those recorded by measurements of concentration. (d) Prolonged protein deprivation may injure the mechanism for synthesizing serum albumin (see *Sections 5 and 8*). If such injury should occur, the replenishment of albumin during the regeneration period will depend not only upon the suitability of the dietary protein but also upon the time required for restoration to normal of the organ concerned in elaborating the albumin.

The range over which measurements of assay values can be made will obviously depend upon the degree of serum albumin depletion obtained during the preparatory period.[1] In 83 experiments the median fall in albumin was 1.02 gm. per cent with quartiles at 0.87 and 1.21 gm. per cent. The distribution of the values is somewhat skewed because of a tendency for higher initial concentrations to be associated with greater declines. The skewness is removed and the variability somewhat lessened when the decline is expressed as a percentage of the initial level. On this basis the average decline with its standard deviation was 29.2 $\pm$6.2 per cent.

It will be observed that the method of assay as outlined does not prescribe a definite amount of basal ration to be fed during the preparatory period. It has been convenient to prepare the diet in 900 gram portions and to offer this amount to each dog daily, irrespective of variations in size. In the 83 experiments referred to in the preceding paragraph the average energy intake was 65 calories per kilo, but the series includes variations between 50 and 87 calories per kilo. With few exceptions this method of initial feeding, dictated by convenience, has been continued because it has not been possible to discover any relationship between calories fed per kilo and degree of serum albumin depletion. In the above series the coefficient of correlation between calories per kilo and the percentage declines in serum albumin was 0.11. At one time we hoped that dogs which had grown too fat might be reduced to a standard nutritional state by underfeeding during the preparatory period. However, the correlation between calories per

[1] For a method of extending the range see *Section* 6.

kilo and percentage loss of weight is only −0.30. This correlation, although significant, is too low to be of practical help.

Section 5. The regeneration of albumin on an intake of 5 grams of protein per kilo. Before deciding upon the level of protein feeding which would be most useful in differentiating various test foods, a series of 18 experiments was conducted in which the dogs received 5

TABLE IV

Experiments at higher level (5.0 gm. per kilo) of protein feeding

TEST FOOD	DOG	SERUM ALBUMIN PER 100 CC.					RECOVERY
		Initial	Depletion	Regeneration	Loss	Gain	
		grams	*grams*	*grams*	*grams*	*grams*	*per cent*
Beef chuck	8-04	3.57	2.56	2.88	1.01	0.32	32
	9-51	3.98	2.96	3.53	1.02	0.57	56
	B-12	3.84	2.62	3.58	1.22	0.96	79
	7-31	3.65	2.82	3.52	0.83	0.70	84
	5-31	4.22	3.08	4.10	1.14	1.02	89
	7-31	3.77	2.73	3.75	1.04	1.02	98
Casein	7-47	3.60	2.84	3.41	0.76	0.57	75
	5-31	3.50	2.06	3.18	1.44	1.12	78
	3-36	3.67	2.64	3.47	1.03	0.83	81
	6-35	2.87	1.94	2.86	0.93	0.92	99
	8-04	2.81	2.13	2.84	0.68	0.71	104
Beef liver	9-49	4.32	3.06	4.29	1.26	1.23	98
	3-36	4.04	3.41	4.14	0.63	0.73	116
Salmon	7-27	3.26	2.30	3.00	0.96	0.70	73
	5-31	3.94	3.07	3.88	0.87	0.81	93
Soy bean	8-04	3.10	1.90	2.58	1.20	0.68	57
	7-31	3.35	2.01	2.87	1.34	0.86	64
Egg white	5-31	4.28	3.04	3.86	1.24	0.82	66

grams of protein and 80 calories per kilo per day. Except for the higher level of protein feeding the procedure in these experiments was identical with that already outlined. The results are listed in Table IV. In six experiments, which dealt with four of the six test foods, the rise in albumin concentration was essentially the same (90 per cent or more) as the loss during the 3-week preparatory period, that is,

the test food produced complete replenishment of the deficit. In six other experiments at least 75 per cent of the loss was regained. Such experiments are not suitable for evaluating the relative potency of different foods, since it is clear that the potency values for a number of the foods would be influenced heavily by the circumstance that regeneration above the normal concentration is scarcely possible.

The results at this high level of protein feeding are important in two respects: 1. They demonstrate that satisfactory regeneration of albumin can be obtained with any of a number of foods if enough of the food is ingested. 2. They demonstrate that the 3-week preparatory period of low protein feeding usually does not result in impairment of the mechanism for synthesizing albumin (see *Section 8*).

Section 6. The nature of the stimulus for regeneration of serum albumin. Previous investigators of the suitability of foods for the regeneration of serum protein have merely assumed that the intensity of the stimulus for regeneration was a function of the degree of serum protein depletion. Melnick and Cowgill (12) developed a "bleeding apparatus" by which it was possible during plasmapheresis to remove exactly the amount of plasma required to lower the serum protein concentration to 3.5 gm. per cent and so maintain a constant degree of depletion. Since there is no proof that the intensity of stimulus increases with degree of depletion, four assays were run in such a way that the relationship could be studied. In these experiments beef chuck was fed at the higher level (*Section 5*), the regeneration period was continued for some time beyond the usual week, and the concentration of albumin in the serum was determined daily. The dogs were large, from 18 to 25 kilos, and not more than 7 cc. of blood was removed for each analysis. The findings are given in Table V. The form of the regeneration curve can be appreciated only by graphing the values; since it is the same for all experiments an average composite graph covering a period of 9 days is presented (Fig. 3). The data lend no support to the belief that the stimulus for regeneration of albumin is a function of the degree of albumin depletion. The stimulus appears to be constant so long as any appreciable depletion exists, that is, the rise in albumin concentration is a straight line function of time. In two experiments, dogs 7–31 and 5–31, the previous albumin level of health was regained. Within the limits of errors of

TABLE V

DOG	BEFORE DEPLETION	DAYS ON REGENERATION DIET												
		0	1	2	3	4	5	6	7	8	9	10	11	12
7-31	3.65	2.82	3.17	2.97	2.99	3.28	3.28	3.65	3.52	3.64	3.63		3.61	3.64
5-31	4.22	3.08	3.04	3.13	3.42	3.50	3.68	3.93	4.10	4.12	4.18	4.11	4.30	4.24
8-04	3.57	2.56	2.58	2.71	2.79	2.81	2.83	2.94	2.88	3.12	3.18	3.14		3.26
9-51	3.98	2.96	3.06	3.09	3.26	3.32	3.31	3.58	3.53	3.60	3.75			
Average...	3.86	2.86	2.96	2.98	3.12	3.23	3.28	3.53	3.51	3.62	3.69			

Results of daily determinations of serum albumin concentration during regeneration on a diet which furnished 5 grams of protein per kilo. The added protein was in the form of beef muscle (chuck). The figures refer to grams per 100 cc. serum.

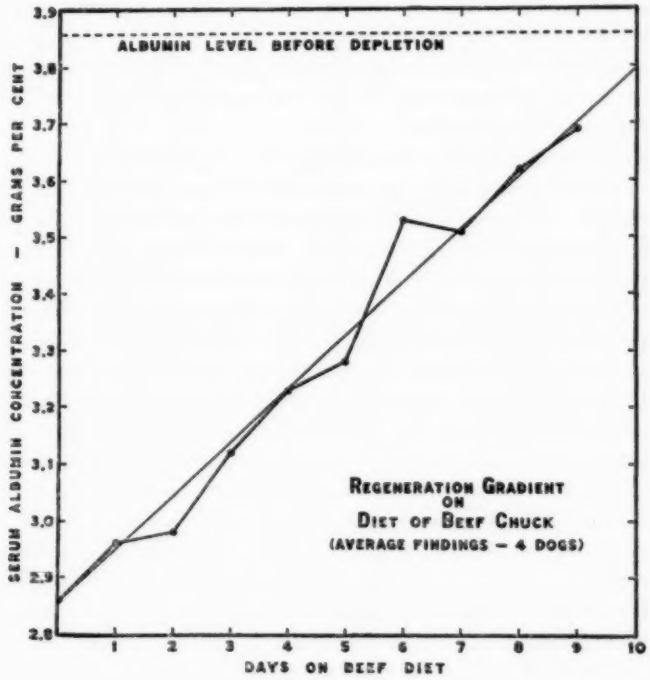

FIG. 3

measurement and sampling (see *Section 9*) the data in Table V show no break in the straight line course of albumin regeneration until this level is reached.

The knowledge gained from these experiments is of peculiar significance in interpreting the results of assays. Since the line of albumin regeneration is straight, concentration rises in the latter portion of the curve are of no greater importance than equal rises in the early portion. Moreover, the finding opens the way for flexibility in the technic for conducting the assays. Thus, the time of 1 week, chosen as a standard period of regeneration, may not prove suitable for evaluating the potency of all foodstuffs. A particularly potent food might conceivably bring about complete replenishment of the albumin deficit within 5 or 6 days, so that the full extent of its potency would not be measured with the standard technic. A comparable potency value for such a food should be obtainable by shortening the period of regeneration to, for example, 4 days and multiplying the rise in concentration by seven fourths.

Upon reflection, it may to some appear unreasonable that the albumin concentration should rise to normal along a path of equal daily increments. Increase in plasma volume must accompany the rising albumin and it is clear that the body would have to synthesize daily larger and larger quantities of albumin in order to bring about equal increments in concentration. Such an adjustment would indeed be remarkable. With this thought in mind we have examined the average findings in Table V as if they were representative of a single experiment. In this average experiment the albumin concentration rose in 9 days from 2.86 to 3.69 gm. per cent, that is, the increment was 0.83 gm. per cent. From this increment a change in plasma volume from 46.2 to 52.8 cc. per kilo was estimated (see *Section 3*). From these figures the total grams of albumin added to the circulation were calculated. Then, on the assumption that the addition took the form of equal daily increments, the figures for albumin concentration and for plasma volume required to yield such increments were computed. The only assumption which enters this final step in the computation is that increases in plasma volume are proportional to increases in albumin concentration; grounds for the assumption have already been presented (*Section 3*). The results, given in Table VI, are compared

with albumin concentrations computed on the assumption that the concentration gradient is linear. It will be seen that the difference between the two values for any one day is always less than 0.02 gm. per cent; both sets of calculated values agree equally well with those found by analysis. The findings in these experiments are, therefore, quite compatible with the thought that the total quantity of albumin added to the circulation daily was a constant. The effect on concentration is a rise which deviates too slightly from linear form to be

TABLE VI

DAYS ON REGENERATION DIET	ALBUMIN PER 100 CC. SERUM			PLASMA VOLUME C.G.
	D	L.G.	C.G.	
	grams	*grams*	*grams*	*cc. per kg.*
0	2.86	2.860	2.860	46.20
1	2.96	2.952	2.960	47.00
2	2.98	3.044	3.058	47.77
3	3.12	3.137	3.153	48.54
4	3.23	3.229	3.247	49.28
5	3.28	3.321	3.339	50.01
6	3.53	3.413	3.429	50.72
7	3.51	3.506	3.518	51.43
8	3.62	3.598	3.605	52.12
9	3.69	3.690	3.690	52.80

Determined and calculated values for serum albumin concentration are compared during regeneration. The figures in column D were determined by analysis. L.G. stands for linear gradient; the figures in this column were computed so as to yield equal daily increments in concentration. C.G. stands for curved gradient; the values for albumin concentration and plasma volume in these columns were calculated so that their product would yield equal daily increments in the quantity of albumin in the circulation. The assumption is made that increments in albumin concentration are proportional to increments in plasma volume.

detected. Moreover, it is clear that even if the deviation could be measured it would *not* denote a decrease in the intensity of the stimulus for albumin formation as the level approached normal.

Although in single experiments of the type under discussion the distinction between concentration gradient and quantity gradient is of negligible significance, it does not follow that this is true under all circumstances. If the rise in albumin were being measured over a range of 2 or 3 grams per cent, appreciable differences might result. Although such ranges are not encountered in these assays, the circum-

stance may nevertheless assume importance when the findings with one dog are compared with those of another. Thus, we may imagine two dogs, both showing a concentration rise of 0.5 gm. per cent and a plasma volume increase from 1000 to 1100 cc. With dog A, however, the initial albumin level was 1.5 gm. per cent and with dog B it was 3.5 gm. per cent. To accomplish the same rise in concentration dog A will have added 7 grams of albumin to his circulation, whereas dog B will have had to add 9 grams. If it is true that the potency value of a test food is more closely related to the quantity of serum albumin which it yields than to rise in concentration, we have here a minor cause of variability among the results of assays performed on different animals. The variability obviously is a function of the absolute values both for albumin concentration and for plasma volume. We have seen, however, that improved consistency is not obtained when measurements of plasma volume are included as part of the method of assay (*Section 3*). The failure may well depend upon the relative inaccuracy of plasma volume measurements as compared with analyses for albumin concentration; suggestive evidence has already been presented (*Section 3*) of an improvement in consistency when calculated rather than determined plasma volumes are used in the estimation of circulating protein. However, we have decided against the use of calculated volumes in computing assay values chiefly because the expression of such values in terms of concentration does not introduce unfamiliar units. At a later date it may become possible to correct each assay value to a value which would have been obtained if the regeneration had been measured from a standard albumin level. Such a correction will depend upon the accumulation of enough experiments to establish a reliable correlation between actual albumin concentration and the magnitude of the regeneration increment.

Section 7. The inclusion in the assay value of a credit for maintenance. Melnick, Cowgill, and Burack (5), who used the method of plasmapheresis to measure potency of foods for regeneration of total serum protein, determined for each test food with each animal the amount required to maintain nitrogen balance. This quantity was subtracted from the amount fed during an assay before a potency ratio was calculated. The six assays which are reported are too few to permit evaluation of the usefulness of the procedure.

In the present work no attempt has been made to distinguish between nitrogen required for maintenance, for the building-up of serum protein, or for the replenishment of other depleted tissues. The patient with hypoproteinemia nearly always presents these same needs concomitantly and it is probably impossible to administer a food which will be utilized for a single purpose. There is therefore a practical advantage in using a method of assay which duplicates many of the features exhibited in human disease. The correction for maintenance which we have adopted, namely, the addition of 0.15 gm. per cent to the increment in albumin concentration, has another purpose. Occasionally a test food will be found which possesses very little potency or which, like gelatin, has no potency whatsoever. During the feeding of such materials the serum albumin will decline rather than rise. Without a correction such foods would be assigned a negative potency value, and a hypothetical food which possessed enough potency to maintain the plasma *in statu quo* would receive a zero value. When dogs are continued on the basal diet during the fourth or usual regeneration week the average decrement in albumin concentration during the week is 0.15 gm. per cent. The addition of this figure to all positive or negative increments will provide a correction such that in averaging a number of assays a potency value of zero will actually mean no potency at all in promoting albumin formation.

In deciding from a small number of assays whether or not a test food possesses any value, it is possible to apply a more sensitive test than the above. The size of the decline in albumin concentration during the fourth week of feeding the basal diet depends to a considerable extent upon the level reached at the end of 3 weeks. The greater the serum depletion after 3 weeks, the less will be the fall during the fourth week. If A equals the albumin concentration after 3 weeks, then Δ_A, the expected decrement during the fourth week, can be estimated from the regression equation:

$$\Delta_A = 0.144\ A - 0.18 \text{ gm. per cent}$$

This equation permits one to compare small decrements in individual assays with the decline which would have ensued if no test protein at all had been fed. Assay values of low magnitude can advantageously be measured from this base line rather than by application of the

standard correction. Obviously the equation could be used to provide a maintenance correction for all potency values irrespective of magnitude. This has not been done because the straight line gradient (*Section 6*) in albumin concentration which is secured on more potent foods would be disturbed by application of the formula and because the addition of a constant correction to all values does not affect the measures of variability of the values.

Section 8. State of the body mechanism for synthesizing serum albumin. Variations among different animals on the same diet in the quantity of albumin regeneration may conceivably result (a) from differences in the amount of protein food utilized for maintenance and for the restoration of other tissues, (b) from differences in digestion or absorption which concern specifically the particular amino-acid mixture needed for synthesis of albumin, and (c) from factors which concern the rate at which individual animals can accomplish the synthesis even when the supply of essential components is abundant. The circumstance that the variations among animals continue to be encountered when the diet contains an abundance of protein (*Section 5*, Table IV), which presumably renders of less relative importance the portions diverted for maintenance or lost during digestion, suggests that the rate at which synthesis can be accomplished is not always the same. When the same animal is used for a number of experiments the findings do not suggest significant variation in the rate at which the synthesizing mechanism is able to function. However, an exception to the rule has served to emphasize the importance of this item. Dog 8-04 has been observed during eight assay experiments. During the first five assays (casein, soy bean, liver, casein, beef) the albumin regeneration was close to the average amount exhibited by other animals which received the same dietary proteins. In three subsequent experiments (beef serum, beef, beef), during which the animal appeared to be in good health, albumin formation was inhibited so that the amount gained was conspicuously less than that observed with any other animal. It is fortunate that in one of these latter experiments we happened to be making daily measurements of albumin concentration (see *Section 6*); the rise in concentration, although slow, followed a straight line gradient as in those cases where the rate of formation was more usual. The behavior of this animal undoubtedly

speaks for pathologic alteration, presumably in the mechanism for synthesis; but since there is no way at present to distinguish between normal and abnormal, it is necessary to accept slightly less marked variations as being within the normal range and to include them when averaging the results of assays to secure a potency value.

Evidence that the mechanism for synthesis may be damaged in another way can also be adduced. When the time of maintenance on the basal low protein diet is lengthened to cover a period of several months, health and vitality are affected markedly, and replenishment of the albumin deficit is sometimes a protracted and difficult process. An example of such delayed replenishment was presented in graph form in an earlier publication (6). With this animal after 85 days on the basal diet the albumin had fallen from 3.9 to 1.4 gm. per cent; the appetite had failed, vitality was low, and it seemed that life could not continue for more than a few days. From this time on an adequate and varied diet was offered daily; because of anorexia small amounts only were eaten at first and life was sustained by gavages of milk. A detailed record was not kept of the amount of food consumed daily but it is clear that the intake of both protein and energy increased slowly as strength and health returned. The behavior of the serum albumin concerns us here. During the first week the rise in concentration was rapid from 1.4 to 2.3 gm. per cent; during the next two weeks the rise to 2.7 gm. per cent was much slower; after three more weeks a level of only 2.9 gm. per cent had been attained. Thereafter, the gains continued but were extremely slow. However, after six months a level of 4.0 gm. per cent had been reached which was slightly above the previous concentration of health. With this animal, after an initial rapid response to food, subsequent albumin regeneration appeared to be independent of the quantity of food consumed. The circumstances were as if one were waiting for restoration to normal of the organ concerned in the synthesis of albumin.

Present opinion inclines to the belief that albumin formation is a function of the liver. After long continued maintenance on the low protein diet dogs will sometimes exhibit evidence of faulty function of the liver by developing jaundice. This is usually a terminal event. At autopsy, however, clear anatomic evidence of injury of the parenchymatous tissue is lacking. Nevertheless, extreme fatty infiltration

and distended bile canaliculi allow one to wonder whether such an organ could function normally.

If one accepts for the moment the possibility that liver injury was responsible for the delayed replenishment of albumin in the experiment just outlined, it is important to note that the injury was one which did not affect the early rate of synthesis but rather that it fixed at a subnormal value the level which could be reached. This circumstance may be of considerable clinical significance for in it one finds a ready explanation for the peculiarly constant levels of serum albumin exhibited by some nephrotic patients during periods when protein intake and degree of albuminuria are varying widely. The fact that such patients may suffer from impaired liver function would account for the failure to observe fluctuations in serum protein when the quantity and quality of dietary protein is varied (Keutmann and Bassett, reference 3).

In the method which has been outlined for assaying food proteins it is unlikely that the second type of injury to the mechanism for synthesis plays a significant part. Depletion of the proteins of body and serum is allowed to continue for only 3 weeks; evidence has been presented (*Section 5*) which suggests that the synthesizing mechanism is generally not impaired during this period. Moreover, in a large number of instances dogs which have completed one experiment have been used for another assay after an interval of 6 weeks of adequate feeding. In such cases the serum albumin has always been found to have returned to the level of health. Such considerations have obviously furnished a cogent reason for using a short period of depletion in the assays.

The real importance of the fact that damage may occur to the mechanism for synthesizing albumin lies in the field of clinical application of the results of the assays to the preparation of diets for patients. Because it is probable that such damage may occur in the human after protracted protein depletion in the same way that it occurs in the dog, it will not be astonishing if many of these patients fail to respond to the feeding of proteins with high potency values.

Section 9. Statistical analysis of the errors of assay.[2] Attention has

[2] In the statistical calculations of this section we have followed chiefly the methods of A. R. Fisher (13).

been called to the biological variation in albumin synthesis exhibited by different animals receiving the same diet. While much of the variation arises from factors which cannot yet be appraised, it is worthwhile to express the total variation in mathematical form in order that the importance of the few known causes of variation can be evaluated. The average total variability has therefore been calculated from 47 assays with 7 different test foods. The result shows an average standard deviation of the assay values, σ_{total}, of 0.169. In this calculation there are 40 degrees of freedom so that the result possesses the same accuracy as would have been obtained if 41 assays with the same test food had been available. The σ_{total} of 0.169 obviously includes the error of chemical analysis, $\sigma_{chem.}$, which has been given previously as 0.030. This error is practically negligible so far as its effect on σ_{total} is concerned; if the error of chemical analysis were entirely removed σ_{total} would still have a value of 0.166 ($\sqrt{0.169^2 - 0.030^2}$).

In the experiments (*Section 6*) in which daily analyses of serum albumin concentration were made throughout the period of regeneration, the summation of evidence indicated that the course of the concentration curve could be represented by a straight line. Nevertheless, many of the individual analyses deviated from the line by an amount greater than could be accounted for by the error of chemical analysis. We have imagined that such deviations might be caused by regular physiologic variations in the volume of the plasma which must occur in response to variations in fluid intake and other factors. Regardless of origin, however, they represent an error in the measurement of assay values, since it is purely fortuitous whether the routine analyses on the first and last days of the regeneration period are somewhat above or somewhat below the line that marks the most probable course of the regeneration. This error may be regarded as a sampling error; it can be evaluated from the 44 analyses in Table V. By the method of least squares the line showing the most probable course of albumin regeneration was calculated for each of the 4 animals. The squares of the deviations of determined values from the lines were then used in calculating the standard error of sampling. In this calculation two degrees of freedom are lost for each of the 4 experiments so that the final result can be thought of as possessing the same accu-

racy as if 37 serial observations had been available in one animal in which the circulating albumin was constant. This standard error is 0.097 gm. per cent. Since two determinations, each possessing the same error, are involved in calculating an assay value, the sampling error of the assay value, $\sigma_{samp.}$, will be 0.138. This $\sigma_{samp.}$ is large and would appear to be of considerable importance in accounting for the σ_{total} of assay values which has been given as 0.169. If such errors could be eliminated σ_{total} would be reduced to 0.098.

The intimation from the foregoing calculation that a large part of the variation among potency values results merely from fortuitous circumstances concerned with the time at which samples of serum are taken from individual animals at once raises the question of whether or not the sampling error can be removed. It is legitimate to inquire what improvement in the consistency of assay values might be expected if the final result were calculated from daily analyses during the regeneration week rather than from determinations at the start and end of the period only. Under such circumstances eight rather than two analyses would be involved in estimating the assay value, that is, the error due to sampling should be halved, ($\sqrt{2/8} = \frac{1}{2}$). Correction of σ_{total} for this increase in consistency leaves a value of 0.112. The practical significance of this result is that the average potency value of a food if calculated from 5 assays by the method of this paragraph should possess the same accuracy as if computed from 12 assays by the method now being employed.

The implication of the preceding calculation can be accepted at this time only as a guide toward the planning of future experiments. Aside from the circumstance that the errors have necessarily been computed from small numbers of experiments, several assumptions, still unproven, have been involved. Thus in estimating σ_{total} the data from assays on a number of different foods were combined, on the assumption that the true sigma of assay values is the same for all foods. Again, although the estimation of $\sigma_{samp.}$ involved 44 analyses, these analyses concerned only 4 animals; if this error varies, as it well may, among different animals, the data are too meager to beget confidence in the result. The calculations are justified only because any suggestion concerning the reason for variability among assays may prove helpful in the design of future experiments.

Even if the foregoing estimate of the error of sampling is accepted at its full value, there remains sufficient biologic variation among different animals to warrant inquiry into its origin. The data, however, are still too few to permit profitable continuance of the statistical inquiry. We may merely mention that several possibilities are being investigated. One of these has already been indicated (*Sections 3 and 6*). In addition, an attempt is being made to learn whether a relationship exists between the rate at which the serum is depleted during the period of low protein feeding and the rate of subsequent regeneration. Finally, we have wondered whether the system of regulating quantity of food on the basis of weight is sufficiently uniform for all animals and with this in mind a study is being made of the relationship between albumin regenerated and the amount of food actually fed per square meter of surface area.

SUMMARY

A method is described for assaying food proteins with respect to their potency in promoting the formation of serum albumin. Depletion of serum albumin is induced in dogs by feeding a basal low protein diet. The diet is supplemented by adding the test food and the rise in albumin concentration during a standard period is measured. The potency value of a test food is the average of a number of assay values from experiments with different animals.

As illustrative of the type of data yielded by the method, the results of a dozen assays of casein are presented.

Measurements of plasma volume, analyzed to show the relationship between albumin concentration and circulating quantity of albumin, do not improve the consistency of the results.

An analysis of the behavior of the globulin fraction fails to show that diet is an important factor in influencing the formation of globulin.

The nature of the stimulus for regeneration of serum albumin is discussed. When the body mechanism for forming the protein is normal, the course of regeneration on a constant diet can be represented by a straight line until the level of health is regained. No support was found for the assumption that the intensity of the stimulus is proportional to the degree of depletion. After prolonged depletion of body protein the course of the regeneration curve may be

altered. Under such circumstances initial regeneration is rapid until a subnormal level is reached; subsequent regeneration is extremely slow and appears to depend not on diet but on the time required for restoration to normal of the organ concerned in synthesizing albumin. Similar circumstances in human disease may limit the effectiveness in the clinic of diets composed of foods of high potency value.

A statistical analysis is presented of the errors involved in making the assays.

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DIETARY PROTEIN AND THE REGENERATION OF SERUM ALBUMIN

II. Comparison of the Potency Values of Beef Serum, Beef Muscle and Casein

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In the first paper of this series (1) a method was described for appraising the potency of various food proteins with respect to their ability to promote the formation of serum albumin. Depletion of serum albumin is obtained in dogs by maintenance on a low protein diet; the regeneration induced by feeding a test food under standard conditions is measured. A single experiment yields a result which is termed an "assay value". The "potency value" of a test food is the average of a number of assay values from experiments with different animals. It is the purpose of this paper to compare the potency values of beef serum, beef muscle, and casein.

DIETS

The composition and preparation of the basal low protein diet has already been described (2); the use of the basal diet in the assay experiments has also been discussed (1). The diet during the regeneration period consists of a mixture of basal diet and test food in such proportions that an animal can be given 80 calories and 2.5 grams of protein per kilo of body weight. Equations have been given which facilitate calculation of the components of the mixture. It is necessary to describe here only the test foods.

Beef serum. Fresh defibrinated beef blood was obtained from the abattoir several days before needed. The serum was separated by centrifugation and analyzed for protein in the usual way. The average protein content of the serum was 7.35 gm. per cent with an average A/G ratio of 1.05. The amount to be incorporated in the regeneration diet was calculated separately for each batch

of serum; in making the calculation the sugar and fat of the serum were neglected. The mixture furnishes 81.8 per cent of the total dietary protein as beef serum protein. The required amount of serum was mixed with the basal diet at the time of feeding. Because of the high salt content of serum the basal diet was prepared for these experiments without inclusion of the usual salt mixture.

Beef muscle. Chuck beef was obtained in ground form from a coöperative butcher who was willing to select for our purpose meat of a constant degree of leanness. Several samples were analyzed for nitrogen; the results showed an average protein content (6.25 × N) of 19.65 per cent. This figure is close to the range of values for lean chuck beef tabulated by Atwater and Bryant (3). The same tables give a fat content from 7.7 to 8.7 per cent; the regeneration diet for

TABLE I

Assays of beef serum for albumin formation

DOG	SERUM ALBUMIN PER 100 CC.					ASSAY VALUE
	Initial	Depletion	Regeneration	Loss	Gain	
	gram	gram	gram	gram	gram	
1-68*	3.39	2.61	2.80	0.78	0.19	0.42
5-31	4.14	3.23	3.66	0.91	0.43	0.58
3-0	3.69	2.83	3.28	0.86	0.45	0.60
7-31	3.58	2.53	3.02	1.05	0.49	0.64
9-51	3.47	2.59	3.10	0.88	0.51	0.66
2-05*	3.63	2.29	2.71	1.34	0.42	0.74
6-35	3.26	2.42	3.11	0.84	0.69	0.84
9-52	2.61	1.45	2.20	1.16	0.75	0.90
3-36	3.72	2.74	3.63	0.98	0.89	1.04
1-69*	3.02	2.19	2.88	0.83	0.69	1.12
1-55	3.61	2.39	3.51	1.22	1.12	1.27

Potency value: 0.801 ±0.053 (P.E.).

* In these experiments the regeneration period lasted for 5 instead of 7 days. Calculation of the assay values is explained in the text.

these experiments was calculated on the basis of 8.0 per cent fat. The components of this diet in terms of amount fed per kilo were: for the basal diet, 47.30 grams; for the beef muscle, 10.67 grams. With this mixture 83.8 per cent of the total protein of the diet is in the form of beef muscle. The required amount of beef was mixed with the basal diet just prior to warming and feeding.

Casein. The results of analysis of the casein and the amount incorporated in the regeneration diet were given in *Section 1* of the previous paper (1).

EXPERIMENTAL FINDINGS

The analytical data and assay values obtained in eleven experiments with beef serum and in twelve experiments with beef muscle are pre-

sented in Tables I and II. Similar data for casein have already been given (1). In three of the experiments with beef serum the regenera-

TABLE II

Assays of beef chuck for albumin formation

DOG	SERUM ALBUMIN PER 100 CC.					ASSAY VALUE
	Initial	Depletion	Regeneration	Loss	Gain	
	gram	*gram*	*gram*	*gram*	*gram*	
1-68	3.92	3.23	3.39	0.69	0.16	0.31
1-69	2.90	2.06	2.28	0.84	0.22	0.37
5-7	3.58	2.60	2.83	0.98	0.23	0.38
5-31	4.17	3.24	3.48	0.93	0.24	0.39
8-04	3.09	2.47	2.72	0.62	0.25	0.40
3-36	4.14	3.28	3.59	0.86	0.31	0.46
9-49	4.08	2.80	3.13	1.28	0.33	0.48
6-35	3.61	2.50	2.89	1.11	0.39	0.54
2-05	3.89	2.48	2.87	1.41	0.39	0.54
7-31	3.80	2.86	3.28	0.94	0.42	0.57
B-12	3.50	2.47	2.91	1.03	0.44	0.59
1-55	3.72	2.89	3.41	0.83	0.52	0.67

Potency value: 0.475 ±0.021 (P.E.).

TABLE III

The behavior of individual animals in assays of beef serum, beef muscle, and casein

DOG	ASSAY VALUE		
	Beef serum	Beef chuck	Casein
1-68	0.42	0.31	
5-31	0.58	0.39	0.36
3	0.60		0.47
7-31	0.64	0.57	
9-51	0.66		0.41
2-05	0.74	0.54	
6-35	0.84	0.54	0.39
3-36	1.04	0.46	0.58
1-69	1.12	0.37	
1-55	1.27	0.67	
5-7		0.38	0.18
Bol		0.40	0.07
8-04		0.40	0.40
9-49		0.48	0.49

tion period was shortened from the usual week to 5 days. So that these data would be comparable with the others, the resulting incre-

ments in albumin concentration were multiplied by seven-fifths before adding the constant 0.15 to obtain the assay values. The rationale of this modification has already been discussed (1). The potency value (average assay value) for beef serum with its probable error is 0.801 ±0.053; the potency value for beef muscle is 0.475 ±0.021. The potency value for casein has already been given as 0.388 ±0.027; this value was based on twelve assays. The probable error of the potency value of beef serum is appreciably greater than that for either beef muscle or casein. The greater variation encountered among the assay values for beef serum may be only a chance happening; it may also be that greater variability is to be expected among the assay values of foods of high potency.

In many instances the same dog has served for assays of two or all of the three foods. For convenience the data regarding the behavior of individual animals are grouped separately in Table III. It is of interest that no animal used for the assay of beef serum gave a higher assay value for either beef muscle or casein. Beef muscle was more potent than casein in three instances (dogs 6–35, Bol, and 5–7), less potent in one instance (dog 3–36), while in three instances (dogs 8–04, 9–49, and 5–31) the results were identical within the error of chemical analysis.

DISCUSSION

One of the important advantages of the present method of assay, which being simple permits of a number of experiments with each food, is that measures of variability are available and can be utilized to test the significance of a difference in potency between any two foods. Such statistical comparison is in this case absolutely essential because of the variability exhibited among replicate assays. The calculations which we have made are based on the method of Student as elaborated by Fisher (4) and take full account of the small numbers of observations in the groups being compared. The values of the probability integral corresponding to calculated values of t have been ascertained from the more extensive tables in the volume by Treloar (5). In interpreting the calculations we shall follow the custom prevalent among those who work with biological data of regarding a difference between two averages as significant when the probability integral is

0.05 or less; when the value of the integral is 0.01 or less the difference will be considered highly significant. The probability integrals (P) calculated on comparing the foods discussed in this paper are presented in Table IV. It is clear that the differences between beef serum and beef muscle and between beef serum and casein are highly significant; a significant difference between beef muscle and casein has not been demonstrated.

For the three proteins under consideration Whipple and his associates (6) have already reported values whch describe potency with respect to formation of total serum protein. Their "potency ratio" is given in terms of the grams of test food protein required for the formation of one gram of serum protein; foods of high potency, therefore, have low potency ratios. These ratios were: beef serum, 2.6; skeletal muscle, 5.7; casein, 10.0. Each value was computed on the

TABLE IV

Tabulation of the statistical probability (P) that the difference in potency value (P.V.) between any two of the foods is significant

FOODS	P.V. DIFFERENCE	P
Beef serum and beef chuck	0.326	0.0007
Beef serum and casein	0.413	0.0001
Beef chuck and casein	0.088	0.0981

basis of a single assay. From data in their protocols we have calculated similar ratios for the albumin fraction. The ratios are: beef serum, 4.8; skeletal muscle, 12.4; casein, 24.2. The relative order of potency is the same as has been found in the present investigation. Comparison of the magnitude of the differences between pairs of foods is not possible both because the methods of assay were different and because measures of variability cannot be calculated for Whipple's experiments.

SUMMARY

Eleven assays of beef serum, twelve assays of beef muscle, and twelve assays of casein for potency in forming serum albumin are compared. The potency values are: beef serum, 0.801; beef muscle, 0.475; casein, 0.388. The differences between beef serum and beef

muscle and between beef serum and casein are highly significant; the difference between beef muscle and casein is not significant.

The order of potency for the three foods is the same as can be calculated from data previously reported by Whipple and collaborators.

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INTRACARDIAC TUMOR PRODUCING THE SIGNS OF VALVULAR HEART DISEASE[1]

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Valvular heart disease is one of the common conditions seen in medical practice. Its cause varies with the valve involved but the two most frequent sources of origin are rheumatic infection and syphilis. In disease of the aortic valve these etiological factors must always be differentiated. However, endocarditis of the mitral valve is so regularly the result of previous rheumatic infection that we accept this sequence of events as true unless the evidence to the contrary is convincing and unimpeachable; and more particularly does this hold for mitral stenosis. There are times, however, when the physical signs of well established mitral disease are present but doubts arise with regard to the rheumatic origin of the lesion. Arteriosclerosis and even congenital lesions may lead one astray, but usually the history or the associated physical findings prevent one from falling into the error of interpreting the signs as those of postrheumatic valvular heart disease. Certain instances of vegetative endocarditis may be confusing, especially those attacking previously unaffected valves but running a subacute course. Metastases to the heart are relatively uncommon and primary malignant tumors of the heart are exceedingly rare. At times, however, primary and benign tumors of the heart, particularly myxomata of the auricular wall, may produce classical signs of mitral stenosis, but here the absence of any history of previous rheumatic infection and especially the appearance and disappearance of the signs with change of position may lead the careful observer to the correct diagnosis. French (1), in 1912, reported an

[1] I am indebted to Dr. William F. Rienhoff, Jr., Associate Professor of Surgery, and to Dr. Thomas S. Cullen, Professor of Gynecology, for permission to report these cases.

instance of antemortem thrombus in the left auricle, producing a ball-and-socket type of mitral stenosis, influencing the action of the mitral valve in a manner identical with that of benign tumors of the auricle. The same author (2) also reports a detached antemortem clot in the right ventricle producing an intermittent stenosis at the pulmonary valve. The signs of insufficiency or of stenosis at the auriculoventricular valves are occasionally produced by the presence of an intracardiac tumor. I am not referring now to chance metastatic lesions in the heart but to tumors entering from the large veins and producing the signs of auriculoventricular valve disease by interference with the closure of these valves. To demonstrate the production in this way of signs of valvular heart disease, and to record two additional instances of this uncommon sequence of events the following cases are reported.

Case 1 (No. 61275). A 51-year-old white woman was admitted to the surgical service of Dr. William F. Rienhoff, Jr. at the Johns Hopkins Hospital, on March 19, 1935, complaining of "pounding" of the heart.

Family history. Unimportant.

Past history. Her general health had been very good, but in 1929, six years before this admission, the left leg had been amputated for a malignant tumor. She had been singularly free from any cardiorespiratory symptoms until the present illness. During the summer of 1934, about ten months before admission, she had an attack of pleurisy in the left side of the chest. This ran a short course and cleared without any unusual incidents. Four months later she began to notice "pounding" of her heart, which was greatly exaggerated by any exertion. This pounding had continued and, if anything, had become worse, but was not associated with pain. Shortness of breath made its appearance at about the same time and became progressively worse. She would occasionally spit a little blood. The patient was examined in November 1934; a mass was found in the left chest and she received a course of x-ray treatments. In January additional x-ray treatments were given. To the latter she had reactions which consisted chiefly of anorexia. This persisted until the time of her admission, and during the intervening months she lost about 15 pounds.

Physical examination. T. 98.6°, P. 100, R. 28, B.P. 100/60. The patient was fairly well nourished; there was no evidence of any great loss of weight. There was slight dyspnea but no apparent orthopnea and she complained chiefly of palpitation. The skin and mucous membranes were of fair color. There was no general glandular enlargement but one freely movable gland was found in the right axilla. Examination of the eyes was negative and the eye-grounds showed no definite vascular changes. The nose and throat showed nothing remarkable.

The trachea seemed to be about in the midline and the thyroid was but little, if at all enlarged. The left chest was fuller than the right and movement was restricted on the left. There was dullness above and below the left clavicle and in the left supraspinous fossa, with diminished voice sounds and suppressed breath sounds. No definite râles were heard. Throughout the remainder of the chest the breath sounds were vesicular and the note was everywhere resonant. There were a few moist râles at the bases, particularly on the right, on deep breathing. The heart was not enlarged to the left but appeared to be slightly so to the right. Dullness behind the manubrium was not increased. The heart sounds were transmitted loudly throughout the left chest and heard quite well in the left back. At the apex there was a short presystolic rumble and a systolic thrill was felt. The thrill could also be felt over the body of the heart but was maximal at the apex. In the left lateral position a soft early diastolic murmur was heard and the second pulmonic sound was decidedly accentuated. The pulses were equal and regular. Examination of the abdomen showed nothing remarkable. Liver and spleen not enlarged and not tender. The left leg had been amputated above the knee, but the scar showed no areas suggesting local recurrence. Specific gravity of the urine varied from 1.012 to 1.025 and the specimens contained neither albumin nor sugar. Microscopic examination negative. Blood on admission: Hemoglobin 80%. WBC 4,600. Wassermann reaction on the blood serum negative. X-ray of the chest: A mass occupied most of the left upper lobe of the lung, which seemed to be entirely independent of the mediastinum.

Two days after admission a bronchoscopic examination was made and with the bronchoscope passed down the left bronchus to its bifurcation no tumor mass was seen and there was no pus or blood. Artificial pneumothorax was induced and on March 27, 1935, an exploratory thoracotomy was performed by Dr. Rienhoff. The left lung was found to be held to the anterior chest wall by adhesions. The tumor in the left upper lobe extended to the periphery of the lung and metastases could be felt in the glands of the hilum. A portion of the tumor was excised for microscopic examination and the diagnosis of spindle-cell sarcoma was made. Following operation the patient was greatly shocked and her condition rapidly became critical. She died four days after operation.

Autopsy (Mar. 31, 1935). The body was that of a well developed and nourished white woman of about 50. There was nothing remarkable about the external appearance except the recent surgical incision on the left side of the chest above the left breast.

Thoracic cavity. The area between the third and fourth ribs on the left side was the site of a recent operation. The upper portion of the left pleural cavity seemed obliterated by adhesions opposite a tumor mass in the upper lobe of the left lung. There were about 300 c.c. of cloudy blood-stained fluid in the upper left pleural cavity and the pleural surfaces were covered with a shaggy fibrino-mucopurulent exudate. The lower lobe was collapsed. Nothing abnormal in the right pleural cavity.

Mediastinal lymphnodes not enlarged and showed no tumor metastases.

Heart. Wt. 275 grams. Epicardial surface smooth. Right auricle and ventricle slightly dilated. The foramen ovale was closed and there were no thrombi in the auricular appendages. Tricuspid and pulmonary valves thin and delicate; the pulmonary arteries contained no thrombi. The left auricle was partially filled by a tumor mass which entered the auricle through the pulmonary

FIG. 1. Case 1. Showing tumor thrombus entering the left auricle from the pulmonary vein and its prolongation through the mitral orifice into the left ventricle.

vein coming from the upper lobe of the left lung. The tumor mass was adherent to the wall of the vein and also to an area on the wall of the auricle just at the entrance of the vein, but beyond this point it hung free of the auricle. It formed a cylindrical mass of firm tissue, the surface of which was smooth and shiny. Just above the mitral valve there was a sharp bend to one side and another bend at right angles, where the tumor turned downward and passed through the mitral orifice. Opposite the mitral valve ring there was a constriction in the tumor

tissue, but it expanded again in the left ventricle and came to an end 3 cm. beyond the mitral valve. The tumor mass was not adherent to any part of the wall of the heart except immediately around the orifice of the pulmonary vein. The whole mass of tumor found within the heart weighed 50 grams. It was about 8 cm. in length and varied from 1 to 3 cm. in diameter. The endocardium of the left auricle and ventricle was smooth and there were no thrombi in these cavities. The mitral valve leaflets were very slightly thickened along the margins of closure but appeared essentially normal. The left auricle was a little dilated; the left ventricle dilated or hypertrophied. The aortic valve appeared normal. The coronary arteries were normal and there was no gross scarring in the myocardium.

Lungs. The upper lobe of the left lung was almost completely occupied by a large tumor mass which measured 12 cm. in diameter. In some areas there was necrosis in the tumor and the pleura over the remainder of the upper lobe was covered with fibrinous adhesions. The lower lobe was free from adhesions but collapsed and the surface of the pleura was covered by a shaggy layer of fibrino-purulent exudate. On the cut surface the upper lobe was found to contain a large rounded tumor mass which left only a narrow zone of intact lung around the periphery. The tumor mass was composed of firm gray tissue arranged in broad bands and whirls. There were many yellowish soft areas of necrosis and numerous dark red areas of hemorrhage. Toward the hilum of the lung, the bronchial and pulmonary veins to the left upper lobe were filled with tumor. In the pulmonary vein the tumor extended along its lumen to the opening into the left auricle and became continuous at this point with the tumor mass in the left side of the heart. In the bronchus the tumor was adherent, from the point of its invasion, for a distance of 3 or 4 cm., beyond which there was a pedunculated mass about 1 cm. in diameter which protruded into the left main bronchus. The peribronchial lymphnodes at the hilum were imbedded in the tumor and had themselves been invaded by it. The lower lobe of the left lung showed dark red atelectatic tissue. There was no consolidation. The right lung showed a smooth pleural surface except for a few fibrous adhesions along its posterior margin, and just beneath the pleural surface were many flattened grayish plaques 2 to 5 mm. in diameter which had the gross appearance of tumor. The peribronchial lymphnodes on the right side were not enlarged and contained no gross tumor.

Abdominal cavity. Spleen, pancreas, liver, stomach and duodenum, intestines and mesentery showed nothing abnormal. The adrenals appeared normal. A single tumor metastasis was found in the right kidney at the inner margin of the cortex. The bladder and pelvic organs showed no gross lesions.

Neck organs. The thyroid was a little enlarged but symmetrical and the normal shape of the thyroid was maintained. The aorta was thin-walled and elastic and contained only a few flecks of yellow fat deposit in the arch and some yellow streaks on the posterior wall of the thoracic and abdominal portions; the inferior vena cava and its large branches contained no thrombi and there was no tumor in the retroperitoneal lymphnodes.

Bone marrow. Appeared grossly normal.

Microscopical examination. Heart. The epicardium, myocardium, endocardium and mitral valve were normal. No tumor was found in the wall of the heart.

Lung. Section through the left upper lobe included a portion of the tumor, and the lung in the vicinity of the tumor showed a thickened pleura containing a fine layer of fibrous tissue and pycnotic leukocytes. The alveolar septa were distinctly thicker than normal and closer to one another. A large part of the section was occupied by tumor which formed a highly coherent and compact mass of cells with little or no stroma. The cells showed scant cytoplasm and a vesicular nucleus, usually containing a nucleolus and a few flecks of chromatin. When transversely cut, the nuclei appeared oval or round; in a longitudinal view, as elongated ovals. Occasional mitotic and hyperchromatic figures were seen and the cells showed a tendency to acinar arrangement. Scattered through the tumor were areas of hemorrhage and necrosis. Section through the bronchus and pulmonary artery and vein showed these structures surrounded by solid and partially necrotic tumor and the pulmonary vein filled with a solid mass of tumor which was directly continuous with the wall of the vein. Despite this continuity of growth the outline of the vein was discernible at almost every point and only in one place was the wall obliterated by tumor.

Tumor in the heart. Section through the mass of tumor hanging in the lumen of the left heart consisted of compact cellular tissue resembling that described in the lung. There were a few small blood vessels and scattered areas of necrosis in the tumor. The surface was covered with a very thin pink stratum. No endothelial cells were seen.

Liver. Microscopic examination of sections showed nothing abnormal.

Spleen. Many of the sinusoids were empty and collapsed and gave the spleen pulp a fibrous appearance, although genuine fibrosis was not present.

Kidneys. No tumor was found. The area referred to as "probably tumor" in the gross description was found to be one of necrosis bordered by hemorrhagic and leukocytic zones, probably typical of an infarct.

Case 2 (No. 29295). A 42-year-old negress was admitted to the Johns Hopkins Hospital on the Gynecological Service on February 11, 1924, complaining of pain in the lower abdomen, bleeding and obstinate constipation.

Past history. She had always been quite well. One child, born at 22 years, had died at birth. The menses began at 12 but were quite regular until 8 years before admission.

Pain began about three years before admission; it was always in the lower abdomen, chiefly in the midline, but at times first on one side and then on the other. It was quite severe but she continued to work. After a time the pain disappeared, but would recur at intervals. Six months before admission she began to bleed from the vagina. The menopause had occurred 8 or 9 years before, but for six months prior to admission she had bled almost constantly. She had sensa-

tions of pressure in the pelvis, constipation had become marked and she was having increasing difficulty with micturition. Four months before admission she had an acute illness with a chill and fever at onset and bloody expectoration for two days. Later nausea appeared and she vomited very frequently. After the acute illness in November her legs began to swell and she became short of breath.

Physical examination. T. 98.8°, P. 96, B.P. 210/120. The patient was well developed and fairly well nourished but there was evidence of some recent loss of weight. The skin was clear and the mucous membranes of good color. The head showed nothing remarkable. The pupils were equal and regular and reacted to light and during accommodation. The nasal septum was intact. The pharynx was slightly injected but the tonsils were not enlarged. Many teeth were missing and there was considerable caries of the remaining ones. The thyroid was not enlarged and there were no abnormal pulsations in the neck. The chest was symmetrical but rather barrel-shaped and respiratory movement was limited. Percussion note resonant throughout; breath sounds everywhere vesicular and no râles heard.

Heart. Apex beat in the 6 interspace 10.5 cm. to the left of the midline. Cardiac dullness extended 11 cm. to the left in the 6th interspace and 3 cm. to the right in the 3rd interspace. The rhythm was regular except for occasional extrasystoles. The first sound at the apex was replaced by a harsh rough murmur heard over all over the heart, but best at the apex, and transmitted toward the axilla. The murmur occupied the whole of systole and was thought to be probably presystolic as well. Second aortic sound accentuated. An intense systolic thrill was felt at the apex and over the body of the heart but not at the base. The pulses were equal and regular except for occasional extrasystoles, and the radial vessels were markedly thickened.

The abdomen showed fullness in the lower half but no rigidity. There was a midline scar below the umbilicus. A large firm nodular tumor was felt extending up to within one finger-breadth of the umbilicus. It was slightly larger to the right than to the left and rather fixed in position so that attempted movement of it was painful.

Pelvic examination revealed a brownish yellow discharge. The outlet was marital and the vagina was filled with a firm tumor which seemed fixed in the pelvis. The cervix could not be outlined but was thought to be dilated by the tumor protruding from it. There were several nodules in the vaginal wall. These were soft and in places necrotic. The adnexa were not felt. There was rather marked tenderness and the fixation of the tumor seemed especially firm in the right broad ligament region. There was pitting edema of the ankles, more marked on the right. The reflexes were equal and active.

Laboratory examinations. Blood count: Hbg. 51%; R.b.c. 2,200,000; W.b.c 9,000; PMN 89%; lymphocytes 6%; mononuclear cells 5%; platelets apparently less than normal in number.

Urine. A somewhat fixed level of specific gravity varying from 1.011 to 1.015. Reaction acid. No sugar at any time, but varying amounts of albumin from a

trace to +++. An occasional white cell and red cell in catheterized specimens, but no casts.

Phenolsulphonphthalein excretion: First hour 0 %. Second hour specimen not obtained. Non-protein nitrogen. 129 mgm. %; blood CO_2: 40.4 volume %; creatinin: 4.84 mgm. %; Wassermann reaction on the blood serum: 0.

X-ray of chest. Aorta dilated with slight enlargement of the heart and marked elevation of the diaphragm.

The patient was seen by a medical consultant who found the physical findings of the heart as noted. It was the impression at the time that there was chronic nephritis as well as cardiac hypertrophy with hypertension and the interpretation of the cardiac signs was that they were due to insufficiency of undetermined etiology. It seemed most desirable that the pelvic condition be relieved and preparations for operation were undertaken, although the patient was in poor condition. She was operated on February 25, 1924, and died on the following day.

Autopsy, Feb. 26, 1924. The postmortem was performed ten hours after death.

The body was that of a well nourished and developed negress. There was no evidence of dilatation of any of the superficial veins. A large tumor mass almost completely filled the pelvis. It was nodular and firm, extending about a hand's breadth above the symphysis.

The gross appearance of the *pleural and pericardial cavities* was normal and there were no adhesions nor free fluid in the peritoneal cavity.

Heart. Weighed 370 grams. In its removal a rod-like structure, about 3 cm. in diameter, was found to fit loosely in the vena cava. This structure continued into the right auricle for about 3 cm., or to the edge of the tricuspid valve, where it was rounded and constricted but continued through the valve orifice as a rod about 8 mm. in diameter. It turned below the tricuspid valve, projected through the pulmonic ring for a distance of 2 cm. above the valve, but at this point ended suddenly. Its surface was smooth and was nowhere attached to the vessel wall or endocardium. The tricuspid and pulmonary valves appeared grossly normal and there were no mural thrombi. There was no lesion of the mitral valve except a yellow sclerotic plaque at its base. The aortic valves were thickened. There was no visible scarring of the myocardium.

Lungs. Voluminous with emphysematous margins and several white nodular areas appearing under the pleura. These were circumscribed tumor nodules, white and without visible structure. Similar areas were found in the peribronchial glands.

Spleen. Weighed 80 grams, was firm in consistence and appeared normal.

Intestine. No lesions found.

Mesentery. The mesenteric glands were not enlarged and contained no tumor.

Liver. Weight 1800 grams. Surface mottled in appearance and on section the centers of the lobules dark red in color, often with a yellow opaque fleck in the center about the hepatic vein.

Kidneys. There was moderate hydropyonephrosis on either side. Pyramids

somewhat flattened at their tips and in some areas there appeared to be dilatation inside the pyramid itself. The dilatation of the ureters stopped at the brim of the pelvis and was evidently produced by the pressure of the tumor.

Uterus. A large nodular mass measuring 17 x 19 x 10.5 cm. It had the external appearance of a large myomatous uterus. On section the uterine structure had disappeared. The whole anterior wall and fundus were replaced by tumor tissue and the uterine cavity had been invaded and was somewhat excavated. Its lining was rough and necrotic. Throughout the body were wide opaque yellow necrotic cheesy areas which were homogeneous, coherent and without softening. These alternated with pale yellow grayish areas of uniform

FIG. 2. Case 2. Showing tumor thrombus entering the right auricle from the inferior vena cava and its prolongation through the tricuspid orifice into the right ventricle.

appearance without structure. Similar areas occupied the posterior wall and the parts around the cervix. The broad ligaments, tubes and ovaries, were incorporated in a mass which seemed to have molded itself to the pelvis. There were areas of tumor in the bladder wall which had not broken through the bladder epithelium.

Vena cava and veins. The veins about the uterus formed ridges on its posterior and lateral surfaces. They were filled with pale yellow masses. The iliac and hypogastric veins and their pelvic branches contained worm-like plugs of the same material. As these came together in the lower end of the vena cava, they formed a twisting interlacing mass, the component parts of which could be sepa-

rated one from the other. These could be traced back to their source in the smaller veins about the uterus. The mass continued to be united into a single rod up through the vena cava to the heart. It passed through the right side of the heart as above described and projected into the pulmonary artery. There were no extensions into the renal or hepatic veins or into any of the branches of the vena cava. At no point was the mass adherent to the wall of the vessel and the intima of the vessel was smooth and shiny.

Retroperitoneal glands. There were a few small white glands in the pelvis and along the iliac artery which were homogeneous on section.

Microscopical Examination. Uterus. There was a great deal of hemorrhage and necrosis. It was impossible to say whether a pre-existing fibromyoma was present or not. The tumor was made up largely of spindle-shaped cells arranged in interlacing strands. Some cells were large and had abundant cytoplasm and in others multinucleated mitoses were frequently found.

The tumor cells stained with the van Gieson stain. The portions in the hypogastric vein and vena cava were covered by endothelium—a most extraordinary feature. Beneath this layer of flattened cells was a condensed thin layer of fibrous tissue exactly in appearance like a vein wall except for the absence of muscle. The smooth covering of the tumor indicated that the blood flowed past the tumor in the vena cava, showing clearly the gross lack of adherence to the vein wall. The veins within the tumor mass contained no tumor.

DISCUSSION

In these two cases of intracardiac tumor the signs of valvular heart disease were undisputable and in each case the mitral valve seemed surely to be the site of the lesion. In case 1 the physical signs of mitral stenosis were classical, whereas in case 2 it was felt that one could not go farther than interpret them as signs of mitral insufficiency. In this instance it was not the mitral but the tricuspid orifice which was involved. It must be said, however, that the murmur was so loud and the thrill so intense, that their distribution was wide and it was difficult to locate accurately their site of origin. In both cases the nature of the valve lesion was not clear, since no history of previous rheumatic infection was obtained. There were no signs or symptoms present which might have been interpreted as those of postrheumatic valvular heart disease except the bloody expectoration in case 1. In the first case, the presence of a tumor in the lung, most surely a growth secondary to the malignant sarcoma of the leg six years before, suggested the possibility of extension of the pulmonary growth to the heart but not in so direct a manner as the postmortem examination

revealed. In both cases the function of the involved valve was so interfered with as to embarrass the circulation. It was difficult to say, however, that frank cardiac failure was present in either instance. In case 1 the cardiac rate was rapid and dyspnoea severe, suggesting left-sided heart failure. In case 2, the dyspnoea and oedema suggested congestive heart failure, but there was a huge pelvic tumor present and a profound degree of secondary anaemia. Regardless of the degree of circulatory embarrassment brought about by these tumors the signs created were practically identical with those of well established valvular disease. In case 1, the signs of mitral stenosis were so classical that it seemed almost incredible that they could in any way be produced by the pulmonary tumor and were not the result of structural changes in the mitral valve. In case 2 the circumstances were even more misleading in that the uterine tumor was considered to be undoubtedly a benign fibroma. No possibility of its malignant nature was entertained and nothing had suggested its presence in the inferior vena cava. The presence of the tumor in the heart was in neither instance due to metastatic implantation or even to growth in and along the vein wall but to extension of the tumor thrombus from the entering vein. This lack of invasion of the vein wall no doubt accounted for absence of complete occlusion of the vessel and allowed a certain amount of blood to pass. In the case of tumor thrombi of the inferior vena cava, at least, as Simpson (5) pointed out in his review, as a rule complete occlusion does not occur Under these circumstances the evidences of collateral circulation are usually absent, and the presence of tumor in the vena cava is not suspected. In the case of the pulmonary vein one would expect evidences of pulmonary hypertension which were present in case 1. This with cardiac signs of mitral disease would only strengthen the view of structural change in the mitral valve. Subsequent microscopic examination established the continuity of the vein wall in its entirety in case 2, and in case 1 at one place only was the vein wall invaded by tumor. The signs produced in the heart by malignant tumors extending into it in this manner would of necessity be very variable. They are dependent, of course, upon the extent of the tumor within the heart but, if time permits, they tend to follow the course of the bloodstream through the heart. The auriculo-ventricular valve on either

side would be the first valve to have its function disturbed. It would seem reasonable that having its closure interfered with would most likely produce the signs of stenosis at the valve.

Invasion of the great veins by tumor in the sense of inclusion within the lumen is not a rarity and particularly is this true with the inferior vena cava. Renal, suprarenal and testicular tumors are notorious in this regard. No doubt the direction of the blood flow accounts for the preponderance of extension toward the heart. Once the tumor has penetrated the vein wall, its growth is largely one of prolongation, since the current of blood tends to prevent implantation upon the intima and the establishment of continuity with the vein wall. It is not unusual therefore to find these prolongations of tumor mass so extensive that the heart is reached. It is interesting that complete occlusion of the vena cava is far less common than one would expect and the mildness of vascular disturbance is striking. Pleasants (3), in 1911, reported 18 cases seen at the Johns Hopkins Hospital and in only four of these was an antemortem diagnosis of obstruction of the inferior vena cava made. The experience of Judd and Scholl (4) of the Mayo Clinic was the same. Of 15 cases reported by these authors only one was recognized before death. Simpson (5), who reviewed the subject of tumor-thrombosis of the inferior vena cava in 1925, found that less than half of the reported cases in which clinical data were given showed clinical signs of caval obstruction. In our case 2 the oedema of the legs, more marked on the right side, may have been due to caval obstruction, but there was no evidence of collateral circulation. One cannot be sure, however, that the enormous size of the uterine tumor did not produce an unequal obstruction to the venous return from the legs. Simpson further found that renal and suprarenal tumors were by far the most frequent to extend to the vena cava, occurring in 60% of those in which the primary site of the growth was known. He felt it futile, however, to attempt to further differentiate this group. Next in frequency were the testicular tumors, comprising 20% of the reported cases. In this series the majority were malignant teratomata. Hepatic tumors accounted for 10% of the cases, whereas the remaining 10% were from scattered sources. In any event the uterus is an uncommon site of origin for a tumor invading the vena cava. In one of the cases observed by

Simpson himself, a carcinoma of the pancreas, a myosarcoma of the uterus was present, but it seemed conclusive from the postmortem examination that the latter was not involved in the tumor extension to the inferior vena cava. Among this larger group of tumor thromboses of the inferior vena except in 22 instances the growth reached the heart and extended into the right auricle. In only 6 of these did the tumor mass enter the tricuspid orifice. In the cases reported by French (6), Jacobson and Goodpasture (7) and Kluge this extension through the tricuspid orifice occurred to a remarkable degree. Apparently little attention was paid to the physical signs produced in the heart. Only in Jacobson and Goodpasture's case, an instance of hypernephroma extending into the right auricle and ventricle, were they described in any detail. Here the heart was enlarged to the left and right. SI at the apex was sharp, a presystolic murmur was heard at the apex as well as a long blowing diastolic murmur. These signs are much more suggestive of mitral stenosis and insufficiency and as in our case 2 localization of the signs in the heart may have been extremely difficult. In the case reported by Breus (9) the state of affairs found at autopsy was most extraordinary. Not only were the right auricle and ventricle filled with tumor mass coming in from the inferior vena cava, but a prolongation had extended through a patent foramen ovale into the left auricle and through the mitral ring into the left ventricle. Unfortunately, the signs over the heart before death were not described, nor do we know what physical signs this most unusual condition created.

Not only extension to the heart but even invasion of the lumen of the vessel seems far less common in the case of the superior vena cava. The same may be said of tumors of the lung reaching the heart by way of the lumen of the pulmonary vein. Adler, in 1912, in a review of 374 instances of carcinoma of the lung, found only one instance of such extension. In a series of 90 cases of sarcoma and in 99 cases of malignant pulmonary tumors of doubtful nature no such extensions occurred. In 1932, Mead reported a case of carcinoma of the lung invading the pulmonary vein and extending to the chambers of the heart through its lumen. And in 1935, Johns and Sharpe report such extension to the heart in a case of primary sarcoma of the lung. In this the lumen of the pulmonary vein "was practically filled by . . .

tumor. The tumor lay free in the auricle but appeared to have invaded the intimal lining of the vein. The cavity of the auricle was filled fairly completely by the tumor mass and there was definite obstruction of the auriculo-ventricular valve." The findings on physical examination are, however, not reported in detail and the only reference to the examination of the heart is that "the heart appeared slightly enlarged to the left, with a systolic murmur heard about half way between the pulmonic and mitral areas." In our case 1, there seems no doubt that the pulmonary tumor was secondary to the previous tendon-sheath sarcoma. Yet the paucity of other metastases and the complete separation of the lung tumor from other implantations allowed it to function as a primary growth and its extension to the left auricle through the pulmonary vein was uninfluenced by any extraneous factors. It seems rather a characteristic of tendon-sheath sarcomata that metastases occur late and that such extensions are commonly solitary.

SUMMARY

Two instances of intracardiac tumor are reported, one involving the left side of the heart and the other the right. In one the tumor arose in the lung and entered the left side of the heart by way of the pulmonary vein. In the other a uterine tumor had reached the heart by prolongation through the inferior vena cava and entered the right side of the heart. In both instances the tumor, unattached to the endocardium, extended through the auriculo-ventricular valve, seriously interfering with its functioning. In one case the classical signs of mitral stenosis were produced, while in the other the signs were interpreted as those of mitral insufficiency.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Major Endocrine Disorders. By S. LEVY SIMPSON. 184 pp. Illus. 10 s. 6 d. (*John Bale Medical Publications, Ltd., London, 1938.*)

An excellent, brief, concise review of the present concepts of major endocrine disorders. Clinical diagnosis and therapy are stressed especially. Practitioners, house officers, and medical students should find this work of especial value as a handy reference book. This reviewer regrets the failure of the author to include a brief bibliography at the end of each chapter and feels that the book loses considerable value by the omission.

J. E. H.

Injection Treatment of Varicose Veins and Hemorrhoids. By H. O. MCPHEETERS and J. K. ANDERSON. 315 pp. Illus. $4.50. (*F. A. Davis Company, Philadelphia, 1938.*)

This book brings up to date a summary of the accumulated knowledge on the injection treatment of Varicose Veins and Hemorrhoids. However, its scope is more comprehensive than the title implies, in that there are also chapters on the embryology, anatomy, and pathology of these conditions, as well as a brief discussion of the operative treatment of varicose veins. The treatment, its indications, and contraindications are outlined in the minutest detail. There are also useful chapters on the complications and causes of failure of the method. In these chapters the enormous experience of Doctor McPheeters is invaluable. The operative attack on varicose veins is discussed generally but not in detail, while in the section on Hemorrhoids it is entirely omitted. The history of the subject and an excellent summary of the literature are included.

The book is of considerable value to the practicing general surgeon, and for him it is to be recommended. It is not, however, a complete monograph on either Varicose Veins or Hemorrhoids—a fact which might be overlooked.

R. T. S.

The Traffic in Health. By CHARLES SOLOMON, M.D. 393 pp. $2.75. (*Navarre Publishing Company, Inc., New York, 1937.*)

This book is similar in scope and subject to many that have appeared in recent years designed to enlighten the consumer about dangerous and fraudulent foods, drugs and cosmetics. It differs from previous similar works, in being a popular treatise on drugs and cosmetics written by a physician. The facts, as stated in it,

appear to be well authenticated. The general purpose of the book and the author's answer to the present deplorable situation which exists in the matter of drugs and cosmetics can be readily seen from the following quotation. "The only way permanently to eradicate the harmful proprietary medicine and cosmetic racket is to promote education. A truly educated public will not so easily be gulled by the producers of nostrums and harmful cosmetics. It is the mission of this book to assist in a small way in that public education. In order to do this the author will ruthlessly tear the veil of mystery from the magic cure-alls and expose their fraudulent nature."

E. K. M.

The Treatment of Clinical and Laboratory Data. By DONALD MAINLAND. 340 pp. 15 shillings. (*Oliver and Boyd, Edinburgh and London, 1938.*)

The author who is a statistically-minded anatomist has prepared this book for the express purpose of teaching the elementary methods of statistical technique, especially those applicable to small samples. He discusses and demonstrates the application of (a) the concept of chance deviations, (b) the chi-square test, (c) the analysis of variance, and (d) correlation and regression statistics.

The illustrative problems are well chosen and represent a good selection of the statistical questions which arise in medical research. The analytical technique employed is appropriate, and in general practical. However, considering that the book is written for the benefit of persons untrained in statistics, some parts of the discussion, particularly those regarding the more complex forms of analysis, should have been clearer and more explicit. At times the explanation of important theoretical points has been limited to a reference to some other textbook. Such a practice is very annoying, since without a thorough understanding of the significance and limitations of a given formula, the beginner will be unable to apply it with the certainty of being able to appraise the results correctly.

A. C.

Symptoms of Visceral Disease. By FRANCIS MARION POTTENGER. 5th ed. 422 pp. Illus. $5.00. (*C. V. Mosby Company, St. Louis, 1938.*)

This fifth edition has been considerably enlarged, and according to the author has been practically rewritten. Amply illustrated, it is an interesting and in many ways a distinguished work. The new chapter on visceral pain is well done. Unfortunately, the author has not kept pace with recent studies of the autonomic system, and has relied for much of his information on other books. To those who have an intimate knowledge of the literature dealing with the innervation of any system of visceral organs, Pottenger's presentation may seem inadequate. It has been conclusively proved, for example, that mammalian striated muscle is not innervated by autonomic nerves. The surgical treatment of diseases of the autonomic nervous system has added considerable physiological information, if it has not always benefited the patient. This is not given adequate attention. The

theory of antagonistic innervation by sympathetic and parasympathetic components can well be challenged.

The book has been written as an introduction to the subject, and from this point of view it can be recommended.

O. R. L.

An Introduction to Pharmacology and Therapeutics. By J. A. GUNN. 5th ed. 240 pp. (*Oxford University Press, London and New York, 1936.*)

The fifth edition of this small book is little changed from the fourth except to bring it in accord with the Eleventh Decennial Revision of the United States Pharmacopoeia. The book is by no means a text for the student studying pharmacology, but designed to give a beginner "a reasoned short account of the scope and matter of the subject, without being a mere catalogue of facts." It serves this purpose well.

E. K. M.

Practical Bacteriology, Haematology and Animal Parasitology. By E. R. STITT, PAUL W. CLOUGH and MILDRED C. CLOUGH. 9th ed. 975 pp. Illus. $7.00. (*P. Blakiston's Son & Co., Philadelphia, 1938.*)

"Stitt" has long been recognized as a mine of information. The present, ninth, edition has been rewritten in such a way that the original value of the work has been preserved and a great deal of additional useful material has been added. The section on hematology, particularly, has been thoroughly revised and four excellent colored plates have been added. The same is true of the chapters on fungi, filterable viruses, spirochaetes, rickettsias, immunity and hypersensitiveness, and many other sections. It has been said of this book that were a physician to be stranded somewhere without access to libraries, this would be the one he should choose to take with him. This appraisal is all the more appropriate for the ninth edition.

M. M. W.

Phenomenon of Local Tissue Reactivity. By GREGORY SHWARTZMAN. 461 pp. Illus. $7.50. (*Paul B. Hoeber Inc., New York, 1937.*)

In 1928 Shwartzman described the phenomenon which now bears his name. Upon injecting the skin of a rabbit with a filtrate of B. typhosus, he found he could produce an area of necrosis at the same site after giving a second intravenous injection of the filtrate twenty-four hours later. Since then this phenomenon of local tissue reactivity has created a great deal of interest. The reaction has been elicited in practically every organ of the body and many different agents which produce it have been described. This monograph is a valuable résumé of our knowledge on the subject. In it the author has gathered together a great deal of information derived from his own investigations as well as from those of others. The nature of the phenomenon, its relationship to other immunological reactions

and its applications to clinical medicine are all discussed. The material is well illustrated and documented. There is a foreword by Jules Bordet.

R. H. F. Jr.

The Management of Fractures, Dislocations, and Sprains. JOHN ALBERT KEY, B.S., M.D., and H. EARLE CONWELL, M.D., F.A.C.S. Second Edition. 1246 pp. Illus. $12.50. (*C. V. Mosby Co., St. Louis, 1937.*)

The recognition of fractures and dislocations and the procedures used in treating these injuries are undergoing such rapid changes that textbooks soon become antiquated. Our authors have obviously taken this fact into consideration in the preparation of the second edition. They have not only revised the book as a whole but have introduced many of the newer concepts and therapeutic devices. The recent advances in dealing with fractures of the spine and of the hip are particularly well described. The completeness that characterizes the handling of the subjects, the interesting and intimate literary style, and the numerous excellent illustrations combine to make this book one of unusual merit. For surgeons who do not survey the literature continuously, or who do not have ready access to a large array of journals, this volume will prove to be of real value.

W. I. N.

Materia Medica, Pharmacology and Therapeutics and Prescription Writing. By WALTER ARTHUR BASTEDO. Fourth Edition. 778 pp. Illus. $6.50. (*W. B. Saunders Co., Philadelphia, 1937.*)

The fourth edition of this standard text has been brought up to date by the inclusion of a number of new articles and the revision of many of the older chapters. The material is very well organized and the salient points under each drug are tabulated so as to permit of a rapid survey. Written from the point of view of a clinical therapeutist and directed toward the practicing physician, this book tends to emphasize results of investigation rather than the methods by which the data are obtained. In view of the limited presentation of the experimental background this is not a text to be used in a basic course in pharmacology, but is of great value for supplementary reading, in that it brings into prominence those features of drug action which are of clinical importance.

M. R.

Materia Medica, Toxicology and Pharmacognosy. By WILLIAM MANSFIELD. 707 pp. Illus. $6.75. (*C. V. Mosby Company, St. Louis, 1937.*)

This book supplies a helpful reference to the botanic nature of vegetable drugs. Drugs are classified according to the part of the plant from which they are derived, roots, barks, leaves, seeds *et cetera*, and a technical botanical description of each is provided. Excellent photographs of the essential plant parts illustrate each of the vegetable drugs. The section on toxicology, an outline compend of the symptoms and treatment of poisoning, is too sketchy and superficial to help either in diagnosis or therapeutics.

M. R.

A Biological Approach to the Problem of Abnormal Behavior. By MILTON HARRINGTON. 459 pp. $4.00. (*Science Press Printing Co., Lancaster, Pa., 1938.*)

This book is another verbal attempt to reduce the complexities of human behavior to a very simple basis by the process of wholesale elimination of a vast acreage of empirical data pertaining to well known concepts of philosophy, psychology and physiology. The author starts by discarding quickly all scientific approaches to the problem of abnormal behavior except two—the Motivistic (psychoanalytic) and the Mechanistic (the attributing of all abnormal behavior to the faulty action of an anatomical mechanism). He states that he is a Determinist because "The doctrine of determinism must be accepted, at least as a working hypothesis, not only in the mechanistic psychology which we are trying to build, but in the building of any kind of psychology." With these small difficulties cleared away, the author proceeds to divide his contribution into three parts: psycho-physiology, dealing with the nature and principles underlying human functioning; psycho-pathology, dealing with the way in which these principles go astray; psycho-orthology, to be presented in a subsequent book dealing presumably with therapy. The word *personality* does not appear in this volume as a topic worthy of any consideration. The Ego, the psyche, soul or spirit, is covered by the term Animism which "looks upon behavior as due to impulses or desires arising in a vaguely imagined something-or-other called the mind." The object of the book seems to be to annihilate any fallacies of human experience associated with this motivistic viewpoint. The writer is very clear as to what he does not believe, but seems confused and tedious in the presentation of what he would substitute for mankind's disillusionments. There is nothing in the 459 pages that offers the perplexed physician, psychologist, teacher, or social worker the wherewithal to slake his thirst.

E. L. R.

The Culture of Organs. By ALEXIS CARREL and CHARLES A. LINDBERGH. 221 pp. Illus. $4.50. (*Paul B. Hoeber, Inc., New York, 1938.*)

The development and perfection of a perfusion pump or apparatus by Charles A. Lindbergh for the study of the anatomy and function of living organs separated from the body has put into the hands of Dr. Alexis Carrel in particular and of the scientific world in general a powerful instrument that has untold possibilities. The exact and detailed description of making and setting up the apparatus is too elaborate for the present review but is of great importance for qualified investigators who wish to utilize the method. The detailed accounts of the perfusion media and of the methods of preparing and inserting organs into the apparatus are likewise sufficient.

Interesting and important results already have been obtained with the thyroid gland, the parathyroid, the adrenal, the spleen, the pancreas, lymph node, ovary, kidney and various other organs. The organs remain alive and can be perfused for many days. Their structure and function vary according to the chemical composition of the perfusion fluid. The future possibilities of the method are very

great. The application to entire human organs for the study of their secretory products and of diseased ones that may be removed from the body, cured by suitable perfusion media and replaced again, are some of the breath-taking future applications suggested by Carrel.

W. H. L.

Methods of Tissue Culture. By RAYMOND C. PARKER. 292 pp. Illus. $5.00. (*Paul B. Hoeber, New York, 1938.*)

An excellent book on the methods of tissue culture. It deals with general and special equipment, cleansing and sterilization procedures, saline solutions, various media and their preparation, various types of cultures and the techniques involved.

Emphasis is given to the methods developed by Carrel and his co-workers. This is especially welcome from a laboratory pre-eminent in this work. There are special chapters on Carrel flask cultures, growth measurements, histological procedures, microphotography and microcinematography, procedures which have been highly developed at the Rockefeller Institute.

A chapter on some applications of tissue culture to experimental biology and medicine serves as a good introduction to the notable achievements of the method. A well selected and classified bibliography is included.

A pithy foreword by Carrel contains the following sentence,—"Along with the invention of the dissection of living and dead bodies, and that of microscopical examination of organs, the creation of the method of tissue culture is the most important event in the history of anatomy."

W. H. L.

Hemorrhoids. By MARION C. PRUITT. 170 pp. Illus. $4.00. (*C. V. Mosby Company, St. Louis, 1938.*)

This monograph deals with a subject at least as old as recorded history and yet of active interest today. The introduction of new or revived methods of treatment of this common malady, with consequent controversial differences of opinion, is the particular reason for recent renewal of discussion. Description and appraisal of various methods of treatment—by operation, by injection of chemical substances and by electrical appliances—provide the principal theme of the book and the author has well and fairly presented the facts. If to this reviewer he seems a little too favorable in his estimation of the value of injection methods, that is largely a matter of personal opinion. His condemnation of electrical methods is certainly well justified. Besides the discussion of treatment, there are good concise chapters on the customary subjects of anatomy, methods of examination, instruments, anesthesia, diagnosis, *etc.* The book is well illustrated and written in clear, easily readable, English. A minor omission noted is the failure to mention rectal strictures in the discussion of differential diagnosis between lymphogranuloma and hemorrhoids. On the whole, the reviewer believes this is the best monograph on hemorrhoids he has read, adequately ample yet not diffuse, thoroughly sound and based on wide practical experience.

H. B. S.

Claude Bernard, Physiologist. By J. M. D. OLMSTED. 272 pp. Illus. $4.00. (*Harper and Brothers, New York, 1938.*)

The reviewer's skepticism of any attempt to improve upon Foster's excellent account of Claude Bernard vanished while reading Olmsted's biography. In addition to presenting the material in very readable form, the author has collected new facts and corrected numerous misconceptions concerning Bernard's life.

The book is divided into three sections. In the first, a very adequate description of Bernard's life is given, with many references to original sources of information. The author discusses Bernard's early struggles and disappointments, his mediocre training, his efforts at playwriting while working as a pharmacist's apprentice, his decision to forsake a literary career in order to study medicine, and his relative failure in the competition for internships at the end of his medical course. The turning point in his career came while he was interning at the Hôtel-Dieu. There it was that his skill was noticed by Magendie, who suggested that Bernard become his assistant in the laboratory. After a brilliant career as a physiologist, Bernard's later life was shadowed by misfortune: forced by ill health and the Franco-Prussian War to leave Paris, deserted by his wife and downcast at the death of his son, he retired to the country where he found his only enjoyment in overseeing the gathering of grapes on his ancestral farm.

In the second section, Olmsted has reviewed critically and masterfully Bernard's great contributions in the field of physiology, correlating them with the science of the time, and indicating their affect upon the development of physiological knowledge. In addition to the major contributions, the author discusses numerous little known observations of Bernard in many fields.

The third section dealing with Bernard's speculative contributions is the least satisfying. Only five pages are devoted to the doctrine of the internal environment and little indication is given that Bernard's name will long be remembered in connection with the introduction of this fundamental doctrine of living systems.

Books of this type are needed. In the ever increasing desire to quote the "latest", historical continuity is forgotten. For example, in a recent textbook of physiology the name of Bernard does not appear among the sixteen hundred references. Olmsted has done much to rectify this deficiency by presenting a very readable description of Bernard's discoveries, by discussing their influence upon the science of physiology and by giving an adequate account of Bernard as a man.

C. L. G.

HUMAN ELLIPTICAL RED CORPUSCLES

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In the 33 years since Dresbach's (5) original description of elliptical erythrocytes in man, at least 134 additional instances, in approximately 32 to 35 families, have been reported (15). Elliptical red cells are of peculiar interest to this clinic since one of the first reports on the subject was that of Huck and Bigalow (7) who discovered the trait in a medical student and were among the first to clearly differentiate elliptical corpuscles from sickle cells.

The purpose of the present report is to record 11 instances of this anomaly in three new families, and to describe the results of some studies on the frequency of elliptical red corpuscles in normal and anemic persons.

DEFINITION OF TERMS AND METHODS

It soon became evident on examining the blood of our subjects that well marked differences in the shape of elliptical erythrocytes occur and that all types, from oval to rod-shaped forms, may be seen. The types of cells we observed are shown in figure 1. Erythrocytes of the shapes b, c and d are hereafter referred to as oval forms, while those resembling e, f, g, or h are called rod forms.

In all instances 200 red blood cells were counted and classified by comparing the shapes of the cells under the microscope with those in figure 1.

When duplicate counts were made on drops of capillary blood under vaseline-sealed fresh wet preparations, it was found, as others have reported (4), that in most cases the number of elliptical cells was somewhat higher in the wet preparations than in the dried smears. However, all the counts here recorded are those made on dried and stained blood smears because this type of preparation was available

for all cases, whereas in only a small proportion were wet films obtainable.

Occasional elliptical erythrocytes are not uncommonly found in many blood smears. Therefore, the use of the term, elliptical erythrocyte trait, has been restricted to those instances in which at least 40% of the erythrocytes were elliptical and at least 10% were rod forms.

The presence of sickle cells was ruled out by failure to observe sickling in wet films of blood examined at intervals for as long as 48 hours, and by the presence of rounded ends on the rod-shaped erythrocytes.

FIG. 1. CAMERA LUCIDA DRAWINGS OF ERYTHROCYTES FOUND IN THE BLOOD OF PERSONS HAVING THE ELLIPTICAL RED CORPUSCLE TRAIT

a, represents an approximately normal cell; b, c and d, represent "oval" forms; e, f, g and h, represent "rod" forms.

THREE FAMILIES WITH THE ELLIPTICAL ERYTHROCYTE TRAIT

In the course of the routine study of the blood of M. V., (family K) who was in the hospital because of congestive cardiac failure, an unusually large number of elliptical erythrocytes was discovered. In this negro family, of which twelve individuals in two generations were examined, four members showed a large number of elliptical erythrocytes. All of these individuals with the exception of M. V. were ambulatory and in good health. None showed evidence of anemia (table I). Fewer rod forms were found among the elliptical erythrocytes in this family than in the C family. None of the erythrocytes suggested sickle cells.

Sternal marrow was obtained from M. V. by puncture. The leukocytes were essentially normal. Only 36 per cent of the red corpuscles found in wet preparations of the marrow were oval in form,

although as many as 83 per cent of the erythrocytes in the circulating blood were oval. Only 1.6% of the red corpuscles in the marrow were reticulocytes. Of these 1.4% were round and 0.2% were slightly oval. None were rod-shaped. Of special interest is the fact that all the nucleated erythrocytes were round.

TABLE I

INDIVIDUALS	RBC	Hb	Ht	MCV	MCH	MCC	ELLIPTICAL CELLS		BLOOD GROUP (MOSS)
							Total	Rods	
							per cent	*per cent*	
Family K:									
M. V. (F 41).....	5.10	14.9	46.9	92	29	32	83	45	III
D. V. (F 17).....	4.16	12.0	38.0	90	29	32	80	38	III
S. K. (M 39).....	5.34	17.0	49.0	92	32	35	74	28	III
Family C:									
J. C. (M 68).....	5.17	12.3	38.7	75	24	32	74	29	
D. I. C. (F 17).....	5.50	13.0	42.0	76	24	31	83	33	IV
Family S:									
H S (F 47).....	4.39	13.6	41.3	94	31	33	48	10	
M. S. (F 12).....	5.01	14.5	43.1	86	28	33	70	20	
J. T. S. (M 10).....	4.32	12.6	37.0	86	29	34	40	10	

RBC refers to number of red corpuscles in millions per c.mm., Hb to hemoglobin in grams per 100 cc., Ht to volume of packed cells in cc. per 100 cc., MCV to mean corpuscular volume in cubic microns, MCH to mean corpuscular hemoglobin in micromicrograms, and MCC to mean corpuscular hemoglobin concentration in per cent.

The individuals are grouped by families and identified by initials. In parentheses following these initials the letters M or F are used to indicate sex and the numbers refer to age.

The results of the examination of a second negro family—the C family—are represented in figure 3. Of the eight members studied, four showed numerous elliptical red corpuscles. All of these individuals, whose skin suggested the mulatto rather than the pure-blooded negro, were in good health and none gave any evidence of anemia (table I). The rod-shaped erythrocytes in this family were in many instances unusually long and narrow, so that blood smears from its members could be readily recognized. In no instance, however, was sickling observed, even after 48 hours.

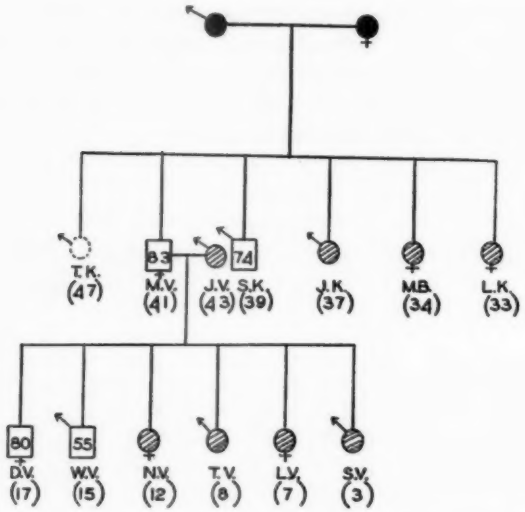

FIG. 2. FAMILY K (NEGRO) SHOWING INCIDENCE OF ELLIPTICAL CELL TRAIT

The open blocks represent those in whom many elliptical cells were found. The proportion of such cells is given within the block. A hatched circle represents an individual whose red corpuscles were not elliptical, an open circle indicates those not examined, and a black circle refers to those dead. Each individual's age is shown in brackets below the initials.

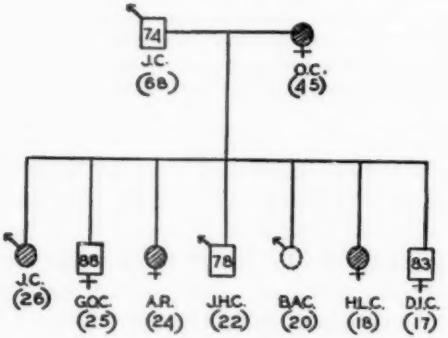

FIG. 3. FAMILY C (NEGRO)

Legend, same as in figure 2

Only three members of the S family, who are white, could be examined. All were found to have numerous elliptical erythrocytes (figure 4) and none showed any evidence of anemia (table I).

Complete blood studies were made in eight instances (table I). In no case did we find erythrocyte counts of 7 and 8 million per c.mm. nor values for mean corpuscular volume as low as 50 and 60 c.μ, as reported by Stephens and Tatelbaum (14). On the contrary, normal values were obtained except in two members of the C family, in whom some microcytosis was observed. It may be pointed out, however,

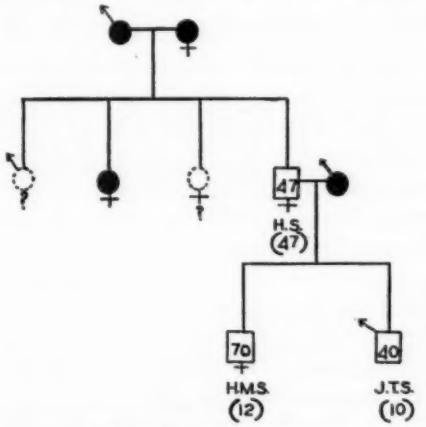

FIG. 4. FAMILY S (WHITE)
Legend, same as in figure 2

that the red corpuscles of these individuals were unusually long and narrow. A difference in the degree of deformity of the red corpuscles may account for the differences between our findings and those of Stephens and Tatelbaum (14). Strauss and Daland (15) also found essentially normal values for mean corpuscular volume, but the erythrocyte counts and hemoglobin values in their subjects tended to be low.

OCCURRENCE OF ELLIPTICAL RED CORPUSCLES IN THE ABSENCE OF THE HEREDITARY TRAIT

A systematic study of the occurrence of elliptical red corpuscles does not seem to have been made. It has long been our impression

that such cells are often overlooked and that they are more common than is generally appreciated. Therefore a study of the presence in the blood of non-anemic and of anemic individuals was made. For the first of these objectives the stained blood smears of 31 medical students and 31 patients from the medical wards were examined.

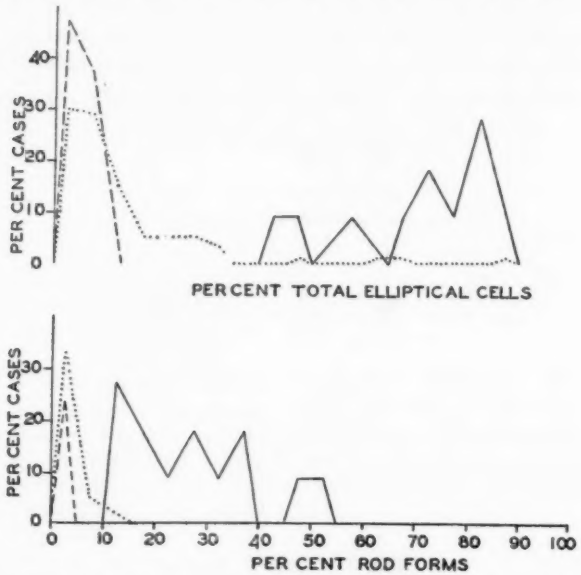

FIG. 5. FREQUENCY OF ELLIPTICAL ERYTHROCYTES IN THE BLOOD OF NORMAL AND OF ANEMIC INDIVIDUALS COMPARED WITH THEIR NUMBER IN THE BLOOD OF PERSONS SHOWING THE INHERITED ELLIPTICAL CELL TRAIT

The interrupted line refers to the blood of normal persons, the dotted line to anemic individuals and the continuous line to those showing the trait.

All forms of elliptical erythrocytes are included in the upper portion of the chart, whereas only "rod" forms are represented in the lower portion.

The patients were unselected except that those without anemia were chosen. Since the findings in these two groups were almost identical, they are represented together in figure 5. Similar observations were then made in 100 consecutive cases of anemia in the files of the Clinical Microscopy Laboratory.

In figure 5 the frequency of elliptical erythrocytes in these two groups is compared with the frequency of such cells in the blood of 11 individuals showing the hereditary elliptical erythrocyte trait. Some elliptical cells were found in 89 per cent of the non-anemic individuals and in 98 per cent of the cases of anemia, but in the former the number of such cells ranged from 1 to 15 per cent, whereas in 12 per cent of the cases of anemia more than 25 per cent of the cells were elliptical. In marked contrast to this, in the individuals in whom the elliptical cell trait was found, the number of such cells was very much greater.

RELATIONSHIP OF ELLIPTICAL ERYTHROCYTES TO TYPES OF ANEMIA

Reference has often been made to the association of anemia with elliptical erythrocytes. Especially in pernicious anemia have oval cells been described (6) and rod-shaped cells have been frequently noted in hypochromic anemia (17). In order to obtain quantitative information, the above mentioned 100 consecutive cases of anemia were studied from this viewpoint and additional unselected cases of hypochromic microcytic and simple microcytic anemia were added to make groups of comparable size.

In 20 per cent of the cases of macrocytic anemia more than 25 per cent of the erythrocytes were elliptical, whereas only 2.5 per cent of the normocytic group, 6 per cent of the hypochromic microcytic and 8 per cent of the cases of simple microcytic anemia, showed as many as 25 per cent elliptical cells (figure 6).

A significant point of distinction between the elliptical cells, found in these cases of anemia, and the cells found in persons with the elliptical cell trait, is that in the former the great majority of the cells are of the oval type. Thus even in the cases of macrocytic anemia in which oval cells were frequently present, only in 5 per cent of the cases were as many as 10 per cent rod forms found. On the other hand, in all persons having the hereditary anomaly 10 per cent or more of rod forms were identified.

These observations suggest that the frequency of oval cells is related to the type of anemia. The degree of anemia, however, does not seem to be an important factor. Figure 7 is a correlation chart relating degree of anemia, as indicated by the quantity of hemoglobin, with the proportion of elliptical cells. Only in the cases of macrocytic

anemia is there any suggestion of correlation. This may possibly be due to the fact that the more common occurrence of elliptical cells in this type of anemia may allow the correlation with the degree of anemia to become more apparent.

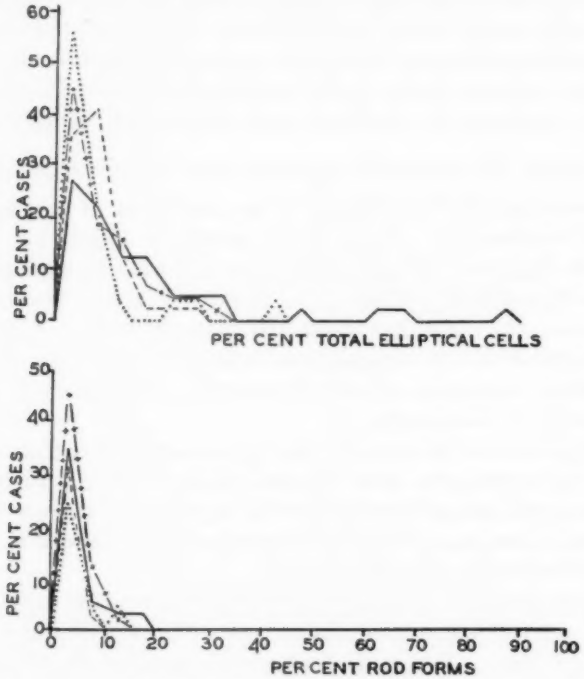

FIG. 6. FREQUENCY OF ELLIPTICAL ERYTHROCYTES IN DIFFERENT TYPES OF ANEMIA

The continuous line refers to the findings in cases of macrocytic anemia, the interrupted line to cases of normocytic anemia, the dotted line to simple microcytic anemias and the line interrupted by crosses to hypochromic microcytic anemias.

DISCUSSION

The red corpuscles of the camel, and those of the non-mammalian vertebrates are characteristically oval in shape. For the majority of erythrocytes in man this shape is very unusual and seems to occur in two forms.

In many of the members of the three families here described, the blood consisted chiefly of elliptical red corpuscles. This has come to be recognized as an inherited anomaly which seems to be transmitted as a mendelian dominant (4, 8, 9). It is unrelated to sex, age, or

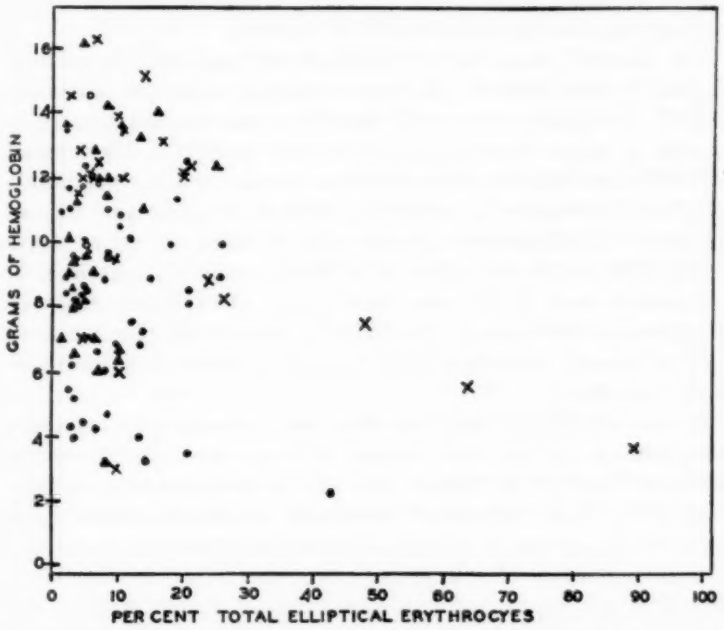

FIG. 7. RELATION OF DEGREE OF ANEMIA, AS REPRESENTED BY QUANTITY OF HEMOGLOBIN, TO PROPORTION OF ELLIPTICAL ERYTHROCYTES IN THE FOUR TYPES OF ANEMIA

× Macrocytic anemia, 25 cases; ▲ Normocytic anemia, 28 cases; ○ Simple microcytic anemia, 24 cases; ● Hypochromic microcytic anemia, 30 cases.

blood group. With one exception (1) the abnormality has never been known to skip a generation.

The elliptical cells which have been observed in cases of anemia differ from those found in the blood of persons harboring the trait in several respects. They are less numerous than round cells and they tend to be oval more often than rod-shaped. The number of these

elliptical cells seems to be unrelated to the degree of anemia, except possibly in cases of the macrocytic type. They have been found to be more common in macrocytic than in other types of anemia, an interesting circumstance in view of the fact that the tendency to develop pernicious anemia, which is the most common cause of macrocytic anemia, is often considered to be inherited.

The physical properties of elliptical red corpuscles have been studied by many workers. It seems to be agreed that the anomalous shape is a property of the cells themselves and not the result of a peculiar or unique influence of their environment (11). Like normal red corpuscles, elliptical erythrocytes are biconcave and, unlike sickle cells, they are rounded at both ends. Elliptical erythrocytes react in the same way as normally shaped cells to hypotonic saline, dilute KOH, dilute acetic acid, dilute HCl, Hayem's solution, lecithin, CO_2, and normal serum of the same blood group. Our observations are in agreement with those of Dresbach (5), who stated that the tendency to rouleaux formation is less marked in these cells than in normally shaped cells.

Human elliptical erythrocytes differ from those of non-mammalian vertebrates in the fact that, whereas the latter are always nucleated, immature forms of the human cells tend to be round. In examinations of the bone marrow of individuals having the elliptical cell trait (13, 16), nucleated elliptical cells have never been seen, and only a small proportion of reticulocytes take this form. In discussions regarding the mature and immature forms of human elliptical red corpuscles, however, the fact has been overlooked that immature forms of the erythrocytes of non-mammalian vertebrates, like those of human instances of ovalocytosis, also tend to be more round than the mature forms.

Inasmuch as elliptical cells are of a distinctive shape which is not affected by the serum of individuals of the same blood group, and since the blood of Huck and Bigalow's (7) patient was used in transfusions without any untoward effect in the recipient, the intravenous injection of these "tagged" red corpuscles by transfusion in studies of the circulation and of the "life span" of blood corpuscles would be of interest. No observations of this kind seem to have been made. Only the statement is made in the report of Huck and Bigalow (7)

that after two months no elliptical red corpuscles were found in the circulating blood of the recipient.

We are indebted to Miss Eleanor White of the Social Service Department for assistance.

SUMMARY

1. "Elliptical erythrocyte trait" is defined.

2. Eleven instances appearing in three families are recorded.

3. A study has been made of the occurrence of these cells in the absence of the hereditary trait.

4. In 89 per cent of a series of 62 non-anemic individuals, between 1 and 15 per cent of the cells were elliptical. In a series of 100 consecutive cases of anemia, 98 per cent of the cases showed many of these cells, in 12 per cent of the cases more than 25 per cent of the cells having been elliptical.

5. Elliptical cells have been found to be more common in cases of macrocytic anemia than in the other types of anemia. In macrocytic anemia these cells seemed to be somewhat more common as the anemia became more severe, but a well-defined correlation could not be demonstrated.

6. The anomalous shape seems to be a property of the cells. This shape is characteristic of the mature rather than of the immature form of red corpuscles of this type.

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CONGENITAL MYOTONIA IN GOATS[1]

Description of the Disease. The Effect of Quinine, Various Cinchona Derivatives, other Alkaloids and Salts upon the Myotonic Symptom

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"Nervous," "stiff-legged" or "fainting goats" were originally described by White and Plaskett in 1904. Since then these peculiarly afflicted animals have been commented upon only six times in veterinary and medical literature. Lush (1930) compared their disorder to periodic paralysis in man, as described by Symonds. Clark, Luten and Cutler (1932) reported blood chemistry studies upon such animals, under the title "A form of congenital myotonia in goats." Levin (1934) in giving one of the best descriptions of the disease, referred to it as "paroxysmal hypertonia." The present study has led to the conclusion that the goats of this particular "nervous" breed are suffering from a form of myotonia which is closely akin to a disorder occasionally seen in man, namely, myotonia congenita or Thomsen's disease.

From the descriptions given by previous observers one learns that, when the goats are startled and make an effort to run away, they suddenly become rigid in all four extremities, and are unable to move. Thus affected, they may remain motionless in a standing position, with rigidly extended legs, for a period of ten to twenty seconds. An animal caught off its balance by the seizure may fall to the ground with the four extremities fixed in extension. After a little while he is able to regain a standing position, but if he attempts to run off immediately, his hind legs may remain stiff for the first few successive leaps. During this time the limbs can be moved passively only with difficulty,

[1] This study was supported by a grant from the John and Mary Markel Foundation.

but once the stiffness has subsided, the animals are entirely free from symptoms for a period of twenty to thirty minutes, after which another attack may be induced. The seizure is not accompanied by loss of consciousness or by clonic movements of the extremities and there is no evidence of autonomic nervous disability. In less severe attacks the muscular rigidity may persist only for an instant, or the animal may do no more than drag its hind legs for a few steps. In almost all cases, however, the animal becomes too stiff to jump over objects a half to one foot in height. It is said that during an attack the diaphragm may become fixed, causing respiration to cease for a few seconds. Isolated reports of animals dying in severe attacks have not been confirmed by any writers upon this subject.

It has been observed that the disorder is inherited through successive generations but no genetic studies have been completed which give any clue as to the manner of its transmission. In a mixed flock of goats, composed of both normal and affected animals, the incidence of the affliction tends to diminish from generation to generation. The young do not usually develop symptoms until some time after birth, although White and Plaskett have observed all the manifestations of the disease in an animal only three hours old.

It seems certain that all of the affected animals in this country are the descendants of a small flock imported into Marshall County, Tennessee. They are now scattered through the states of Kentucky, Alabama and Texas. It may be of interest to quote again a letter concerning the introduction of these animals into Tennessee, written by R. J. Goode of Gastonburg, Alabama, to J. D. Lush.

> About 1880 a stranger came into a community in Marshall County, Tennessee, carrying with him four goats and what he described as a sacred cow. He went to Dr. Mayberry and asked that he be allowed to keep his goats and cow at Dr. Mayberry's place and, in return for his meals and keep for the animals, did odd chores for Dr. Mayberry. He stayed at Dr. Mayberry's place several months and later married an old woman in an adjoining community. He stayed during one farming season and at the end of the year came back to Dr. Mayberry and sold him the four goats. He then left with his cow without telling any one where he had secured the goats or the cow or anything about his personal history.

In speaking of the stranger who introduced the animals into Tennessee Dr. Sam L. Clark tells me "he wore two hats and presumably

the smaller of these was a fez." White observed a flock of similar animals in Syria in 1929. He recognized their peculiarity as the whole flock fell to the ground when the train whistle was blown.

The animals were introduced into Texas purely to uphold the veracity of a Tennessee farmer. Scaring them was once a popular pastime.

The goats of the original flock were snow-white in color, but those now obtained have usually been crossed with Angora or other strains, and they commonly have brownish markings. Although they are easy to handle and catch, since they are unable to run and jump as well as normal goats, their symptom, which is accentuated by cold, often causes them to be frozen to death during severe winters. The difficulty in driving them for shipping or slaughtering, and their "tough red meat" make them undesirable to breeders. Consequently few truly "nervous goats" can now be found.

DESCRIPTION OF EXPERIMENTAL ANIMALS

A four-year-old billy goat and a seven-year-old nanny were obtained on February 2, 1938. Both were said to be "nervous," in fact, they had been used for advertising purposes previously. The nanny was pregnant; she had been crossed with the billy. The billy was long-haired with muddy white or beige markings over the fore quarters, neck and head. The nanny had a large brownish patch over the hind quarters and the left fore quarter but was elsewhere snow-white and short-haired.

Attempts at inducing the rigidity in the nanny were at first unsuccessful. When the billy was chased he made a strenuous effort to leap away which ended in his hind legs becoming stiff in extension and dragging behind him. On other occasions the fore legs also became rigid. He then had the appearance of an immobile statue with the hind legs extending behind and the fore legs projecting in front of the body. Within ten seconds he was able to move away. The first steps were slow and impeded and the hind legs dragged, but he quickly regained his agility and it was difficult to catch him again.

Two kids, a male and a female, were born on February 24. In hair and coloring, the male resembled the billy, the female, the nanny. The kids were not easily frightened, in fact were quite friendly. No evidence of myotonus could be demonstrated at this time.

METHODS

The animals were observed at weekly or biweekly intervals. In preliminary experiments they were driven into a small pen, where they were allowed to rest for half an hour. They were then carried out of the pen and allowed to run back toward it. This seemed to be a fairly effective method of producing a seizure. Later on, a hammock was constructed in which the goat under study was placed for a thirty-minute rest period. When he was released from the hammock, the animal immediately made a strong effort to leap away and join the rest of the flock in the pen. During this effort typical rigor developed. It was not found necessary to frighten the animal by making loud noises or by rushing at him.

It was discovered that the effect of various factors upon the myotonia could be estimated quantitatively by observing the length of the rest period which was required before a seizure could be induced. The standard rest period was 30 minutes. Under ordinary circumstances a seizure always occurred when the animal was released at the end of this time. When the myotonia, or at least the tendency toward myotonia, was increased, seizures occurred after much shorter intervals of rest.

Various drugs were given intravenously and their effects upon the rigidity observed. If it was expected to diminish the myotonia, it was given 10 minutes before the end of the 30-minute rest period. If it was expected to increase the symptoms, it was given at the beginning of the period. The criterion of a beneficial effect was that no rigidity appeared when the goat was removed from the hammock after a 30-minute rest. On the other hand, a drug was considered to have increased the myotonia if an attack could be induced after no more than a 5-minute rest. Using these criteria, the results were clear cut enough to allow one to determine whether the drug had a moderating effect, an aggravating effect, or no effect at all upon the disorder. At the time these tests were made, the billy weighed 45 kg. and the nanny 39 kg. The kids increased in weight from 8 to 10 kg. during the period of the tests.

Cinematographic records[2] were made from time to time to record the growth and varying characteristics of the family, and also to provide a permanent record of the effects of the various drugs.

The reaction of the muscles to percussion and the muscular responses to galvanic and faradic stimulation were studied. By tying a string to the ankle, and running this over a pulley to a counter-weighted writing point, it was possible to record the muscular reactions upon a kymograph.

Biopsies of the distal portion of the lateral belly of the gastrocnemius muscle were obtained from the billy and nanny. The tissue was fixed in formalin, imbedded in paraffin, and sections were cut at 6 to 15μ. The sections were stained with (1) haematoxylin and eosin, (2) iron haemotoxylin—van Gieson connective-

[2] Shown before the first meeting of the Society of Physiological Neurology, Atlantic City, May 2, 1938.

tissue stain and (3) a modification of Cuajunco (1927) silver impregnation method for end plates and muscle spindles.

Two normal goats were subsequently obtained and studied by the methods reported above.

OBSERVATIONS

The myotonus. The nature of the attacks has been previously described, and from a large number of repeated observations we can add little to what has been brought out before. The muscles of the hind limb, particularly those of the calf, appear most markedly affected. Stiffness of the hind legs is seen in every attack; that in the fore legs is much less obvious.

All the previous descriptions have stressed the fact that the attacks may be induced by frightening the animals with some loud noise or rushing upon them waving the arms and shouting. For this reason Levin described the condition as "paroxysmal hypertonia induced by affect." From our observations, using the hammock as a rest, and allowing the animal to run back to the other goats in a pen, it seems quite clear that neither fright nor surprise is the cause of the disorder.

That rest preceding the exertion is an essential factor is shown by the following study. A series of observations were made upon the nanny and the kids by allowing them to remain in the hammock for varying periods of time. It was found that though five minutes complete rest after exercise was not effective in bringing out the symptom in the nanny, after ten minutes rest her legs dragged for a moment. Twelve minutes rest in the hammock was followed by definite rigidity in all four legs as the animal started away. After twenty-four minutes rest, the rigidity which developed immediately after release was so marked that several seconds elapsed before the nanny was able to move. The results with the female kid were much the same. Nine minutes rest had no effect. A period of twelve minutes rest led to rigidity in all four limbs which was quickly overcome. After fifteen minutes rest, as soon as she was released from the hammock she fell to the ground and remained immobile for several seconds. It is interesting that rest up to fourteen minutes was not effective in eliciting the symptom in the other kid. These observations, therefore, give a certain indication of the degree of the disability in each animal and a basis for the interpretation of the reactions in the pharmacologic tests.

Another factor which must be considered is the force exerted in the muscular action undertaken. When a violent effort to escape was

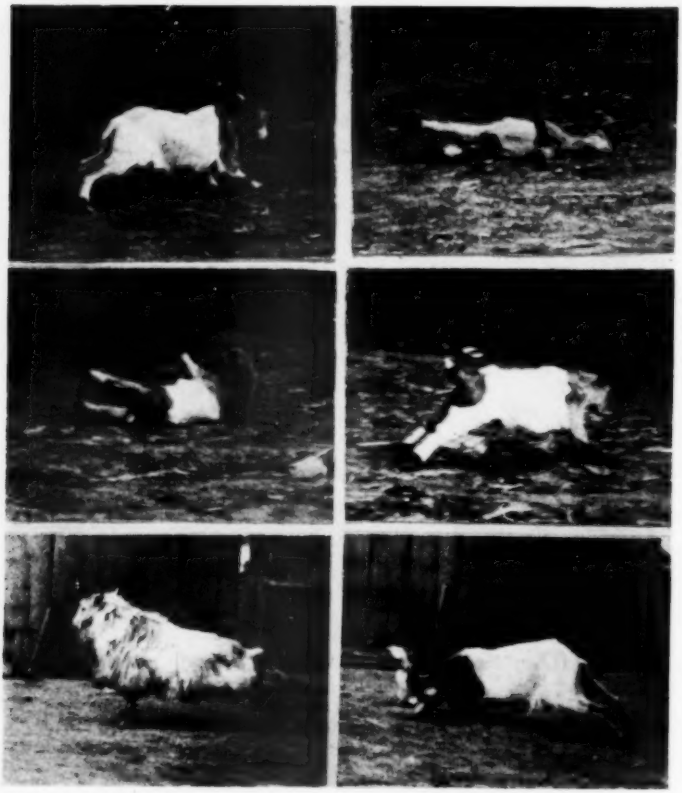


FIG. 1. THE VARIOUS POSITIONS ASSUMED DURING MYOTONIC SEIZURES

The legs were rigid and motionless at the time that these pictures were taken. The upper four photographs are reproductions from the cinematographic film, the lower two are still photographs.

made, the rigidity seemed more marked, and frequently on such occasions the goat fell. It is our impression that the rigidity is accentuated by cold. We have not tested this in any objective manner, but have

observed that the animals appear stiffer on cold days and the rigidity seems to endure longer.

The delayed development of the myotonus in the kids of affected families has been commented upon by others. We were never able to induce any rigidity in the kids during the first month of life. Later they showed symptoms that were even more striking than those of the nanny or billy. This was first clearly seen on the thirty-fifth day of life. The failure to elicit symptoms earlier suggests two possibilities. The muscular defect may have been delayed as it is in Thomsen's disease, or the young may not have exerted their muscles in the forceful and direct manner which produces the prolonged after-contraction. The first possibility seems the more probable.

It is of interest that the nanny failed to show any rigidity during the latter part of pregnancy and for thirty-five days thereafter, although she was reputed to be a "nervous goat" by her previous owners. We were unable to induce an attack in any of the ways described before. She has manifested typical rigidity under appropriate conditions ever since.

Percussion reactions. A characteristic of myotonus in man is the prolonged contraction of a muscle after it has been struck with a percussion hammer. This is usually seen as dimpling in the region where the hammer impinges. This reaction is never noted in normal muscle, which responds with a quick muscular twitch. On smartly striking the muscles of the calf in all four of the affected goats (after the symptom had appeared in the kids and the nanny) a characteristic myotonic dimple appeared and persisted for a period of about five seconds. Such a reaction was not obtained on percussing the muscles in the two normal animals.

Electrical reactions. The reactions of the muscle to stimulation with galvanic and faradic currents were also examined. In man the "myotonic reaction" is characterized by the prolonged contraction existing during the flow of the galvanic current. This is in contrast to the simple muscular twitches seen on the make or break of the current in normal individuals. Following faradic stimulation in myotonic individuals there is a period of delayed contracture after the flow of current has been interrupted.

In a series of observations recorded kymographically we have never

elicited in the goats the myotonic reaction to galvanic stimulation. The responses were simple muscular twitches. With faradic currents of sufficient strength to produce a muscle tetanus, but insufficient to lead to pain, no after-contracture was obtained. With currents leading to pain the animal invariably was startled, struggled, and the limbs became rigid. We concluded that these responses were brought about by a violent voluntary muscular contraction in the animal's attempt to avoid the painful stimulus.

Biopsy studies. A careful examination was made of microscopic sections of the gastrocnemius muscle of one of the normal goats. In longitudinal sections the transverse striations were easily seen, the longitudinal less so. There appeared to be a large number of sarcolemma nuclei along the sheaths and here and there aggregations of nuclei were seen lying upon the fibres. The bundles of fibres were usually closely packed, connective tissue being scanty. Fat was not observed in or around the muscle bundles. On transverse section the average diameter of the fibres was 58μ. The smallest fibre seen in a count of 300 was 33μ and the largest 104μ. Several muscle spindles were seen in these sections. The sheath was not unusual and the periaxial space of average size. Two to five intrafusal muscle fibres were commonly seen. These were on the average 20μ in diameter, and showed centrally placed nuclei and nuclear aggregates in the equatorial region. The nerve endings upon the spindles and elsewhere were not abnormal.

The muscles from the affected nanny and billy were much the same as those of the normal goat. The longitudinal sections displayed well marked transverse striations. Again, as in the normal, the sarcolemmal nuclei were often found massed together (fig. 2). Four spindles were found in the serial sections of the billy goat's gastrocnemius and two were noted in similar sections from the nanny. Nothing abnormal was observed. A few normal appearing nerve endings were identified in these preparations. Measurements of a series of fibres in the transverse diameter gave an average of 63μ. Three hundred fibres were measured in each animal. The largest fibre seen was one 112μ in diameter from the billy, but others as small as 33μ were also present. On the whole the variations in size were no more marked in the affected than in the normal animals. The final conclusion reached was that no readily detectable histological abnormality exists in the myotonic animals.

Pharmacologic reactions. It has been shown by A. Wolf (1936) that the myotonic phenomenon in man may be abolished by quinine, given either orally or by intravenous injection. Later, Kennedy and Wolf (1938) pointed out that prostigmin accentuated the rigidity, and antagonized the effect of quinine. These findings have since been corroborated by Smith (1937) and by Kolb, Harvey and Whitehill (1938). The remarkable effect of quinine in causing the disappearance of the myotonus may be regarded as a diagnostic test differentiating this condition from other forms of muscular rigidity. We have examined, in goats, the effects not only of quinine and prostigmin, but also of various other cinchona derivatives, some alkaloids, certain inorganic salts, alcohol and guanidine.

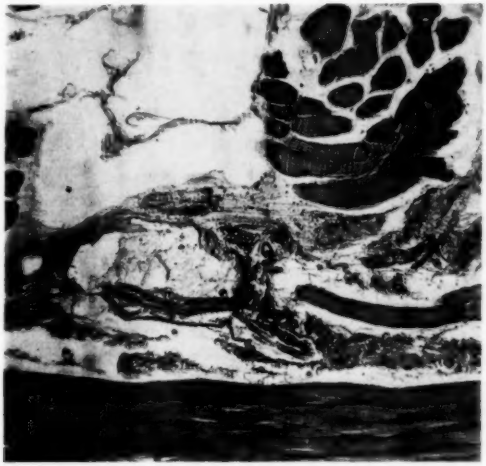


FIG. 2. MUSCLE BIOPSY SPECIMEN FROM THE AFFECTED NANNY. H. & E. × 30

Both longitudinal and tranverse sections of muscle may be seen. A large muscle spindle lies in the central field. With the exception of the numerous sarcolemma nuclei, which are normal for the goat, nothing unusual is seen.

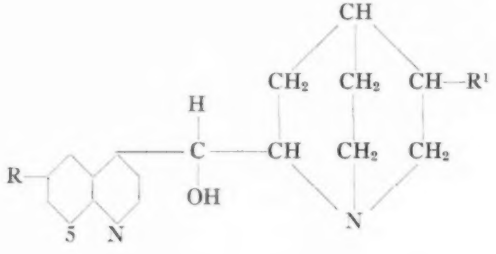
Quinine. This drug was administered as the dihydrochloride intravenously on fourteen occasions. The dose given the adults on each trial was 0.5 gm.; the kids received 0.16 gm. the first time and 0.25 gm. the second time (six weeks later).

Quinine, administered in this manner, leads to a complete oblitera-

tion of the rigidity. As soon as they were removed from the hammock and placed upon the ground, the goats jumped and ran with a speed and agility which was the extreme opposite of their previous awkward and restricted attempts at escape. This was most striking in the kids who actually leaped and cavorted in the air. The quinine effect has been observed to persist up to two hours after administration of the drug, successive tests being made at half-hour intervals. Tests have not been carried beyond this point, but the drug probably acts over onger periods if one may judge from the results in man.

Prostigmin. This drug was administered four times intravenously and six times subcutaneously. The dose given was 2 mgm. to the adults and 1 mgm. to the kids. Following the intravenous injection the rigidity was pronounced after a five-minute rest period in the nanny and the male kid. Within an hour after its subcutaneous administration the animals were usually so rigid that they could hardly initiate movements, an effort lasting for several seconds being required before they were able to move away. It is interesting that in the early period, before myotonus was clearly apparent in the kids or the nanny, we were twice able to induce, by means of prostigmin injections, slight stiffness and dragging of the hind limbs, once in the nanny and once in the female kid. When prostigmin is given forty minutes after an effective therapeutic dose of quinine (0.5 gm.), the rigidity reappears within thirty-five minutes. We have previously mentioned that the quinine effect ordinarily lasts for more than two hours.

Cinchona derivatives. Since numerous other derivatives of the cinchona series have actions similar to those of quinine, it was decided to examine these related compounds in some detail. The general formula for these alkaloids is given below. The various derivatives are summarized in accordance with information given by Dawson (1930).



In the list appended below the first alkaloid of each pair is levorotatory, the second is its dextrorotatory isomer. Where only one is given it is levorotatory.

Cupreine Series, $R^1 = CHCH_2$

R = H, cinchonidine, cinchonine
= OH, cupreine
= OCH_3, quinine, quinidine
= OC_2H_5, ethyl cupreine

Hydrocupreine Series, $R^1 = CH_2CH_3$

R = H, hydrocinchonidine, hydrocinchonine
= OH, hydrocupreine, hydrocupreidine
= OCH_3, hydroquinine, (methyl hydrocupreine), hydroquinidine (methyl hydrocupreidine)

Amino hydroquinine, NH_2, Group at (5)

= OC_2H_5, optochine (ethyl hydrocupreine)
= OC_3H_7, cincane (isopropyl hydrocupreine)
= OC_5H_{11}, eucupine (isoamyl hydrocupreine)
= OC_8H_{17}, vuzin (isoctyl hydrocupreine)

Quitenine Series, R = CH_3O

R^1 = COOH, quintenine, quitenidine

Of the drugs listed above we have tried quinine, cinchonidine, cinchonine, hydrocinchonine, hydroquinine, hydroquinidine, eucupine, quitenine, and quitenidine and also ethyl-hydroxyapocuprein hydrochloride. The cinchonidine, cinchonine and hydroquinidine were administered as bisulphates and the rest as dihydrochlorides; the intravenous dose being 0.5 gm. for the adult and 0.16 for the kids at two months and 0.25 at three months of age. In each case the trials were repeated from two to seven times. All were as effective as quinine in the doses given except eucupine and the carboxyl compounds quitenine and quitenidine which failed to modify the myotonic symptoms. The single trial with hydroquinidine immediately proved fatal to the male kid. Eucupin was the only other drug given which produced any marked side effects; respiration was irregular both in depth and rate.

Atebrin, the synthetic acridine dye compound having potent antimalarial properties, was also tried on six different occasions. The intravenous dose given to the adults was 0.08 gm. twice, 0.1 gm. once

and 0.2 gm. once. Each of the kids received 0.02 gm. of the drug once. The adults never showed any improvement; the two kids were both free from rigidity. The conclusion reached was that the drug was much less effective than the simpler compounds used above.

Alkaloids. Atropine sulphate was given intravenously once to each animal, the adults received 0.0006 gm. and the kids 0.0002 gm. The drug failed to modify the rigidity in any way. Pilocarpine nitrate 0.006 gm. by vein twice brought on the typical rigidity in tests carried out within five minutes after its administration. Cocaine hydrochloride on a single trial was ineffective in a 10 mgm. dose.

Adrenaline hydrochloride 0.5 mgm. given intravenously proved ineffective on two occasions.

Ephedrine sulphate was given to the adults in 25 mgm. doses intravenously and to the kids in 8 mgm. doses. The two adults and the more affected kid were rigid within five minutes after the administration of the drug but after a twelve-minute rest. The other kid showed no change. Twelve mgms. of the drug given to the female kid half an hour after 0.325 gm. of quinine did not produce any rigidity.

Guanidine hydrochloride was tested, as Minot, Dodd, and Riven (1938) have recently reported the effective relief of myasthenia with doses of this drug of 6 to 10 mgm. per kilo. Consequently it was decided to observe its effects upon the caprine myotonus. The adults received an intravenous injection of 0.325 gm. each on one occasion and no definite effect was noted. Of the two kids only the more affected one showed rigidity in a test carried out five minutes after the intravenous administration of 0.16 gm. A second trial with 0.325 gm. failed to bring out the myotonus. This kid was given a preliminary injection of quinine 0.2 gm. on another day and forty-five minutes later received 0.162 gm. of guanidine hydrochloride. There was no reversal of the quinine effect. The nanny received 1.9 gm. of the drug half an hour after 0.5 gm. of quinine, and again the myotonus did not recur.

Inorganic salts. Potassium chloride proved ineffective on four occasions given by vein in 1.0 gm. amounts to the adults and 0.5 gm. to the kids. Two trials of 0.5 gm. of calcium gluconate were negative. A similar negative result was observed after the use of 0.5 gm. of magnesium sulphate. Barium chloride in doses of 0.032 gm. to the adults

and 0.010 gm. to the kids appeared to increase the rigidity in the two adults after a one half-hour rest. Rigidity was not induced in either kid in the five-minute rest period test. On one occasion slight rigidity was evident within five minutes after giving .065 gm. to the nanny one half hour after an effective dose of quinine (0.5 gm.). A previous trial, giving 0.03 gm. to one of the kids after quinine, caused no reversal of the beneficial effect due to the latter drug.

Alcohol. Russell and Stedman (1936) first reported that alcohol reduced the myotonic symptoms. Consequently, we have given the drug in saline on six occasions in doses up to 40 c.c. of 40% alcohol by volume. Such a dose made the animal drowsy and respirations assumed a deep gasping character. In no instance was the myotonic symptom relieved.

DISCUSSION

The descriptions of this disease in goats brought out so many points that are regarded as characteristic of the myotonic state in man that our interest was directed to the present study. The features of importance are the unusual rigidity, its appearance with muscular effort, improvement with further effort, and reappearance or accentuation after rest. Furthermore, the increase in the rigidity with decrease in external temperature is well recognized. As in man, the affliction is inherited and tends to be observed in the young only after a lapse of some time after birth. The effects of pregnancy upon the rigidity have not been studied to any extent in the human disease. It is interesting that, with myasthenia gravis, pregnancy often seems to enhance the weakness. On the other hand, our nanny was free from myotonus for a month following delivery. What influence the various hormones exert upon muscular activity remains to be studied.

The recognition of the symptom is often difficult if the animals have not previousy been seen. In fact it is usual to overlook the disorder at first. Only when the animals have been at rest for some time and are then urged to run is the rigidity obvious. In myotonia congenita also the patient may learn to modify his symptom by undertaking continuous mild exercise and by avoiding those activities which demand great muscular effort after a period of rest.

The specific tests for myotonus, with the exception of the electrical

reactions, support the contention that these animals are suffering from this disorder. The percussion reaction was easily obtained. The rigidity on passive movement has been commented upon before. The failure to obtain electrical reactions similar to those described in the human disease may be due to several causes. The disorder may differ in this respect from Thomsen's disease in man. The method used may not have allowed sufficiently delicate observations. The reactions are often difficult to obtain in man. It is hoped that the problem can be reviewed later with a nerve-muscle preparation and that a more detailed and better controlled study can then be made.

No definite pathologic changes have been described in Thomsen's disease in man though atrophic changes are present in that complex disorder, myotonic dystrophy. The histological studies of the muscle fibres, nerve endings and spindles on the few goat specimens obtained appeared normal. The description of the muscle as being unusually red and tough may be of some importance.

The chemical studies of Clark, Luten and Cutler upon myotonic goats showed no significant changes in the blood sugar, calcium, or inorganic phosphates. No deviations from the normal were noted after the injection of glucose, adrenalin or insulin.

The complete disappearance of the rigidity after the administration of quinine and the aggravation of the symptoms by prostigmin parallel the situation in man. We have found that quinidine, cinchonine, cinchonidine, hydroquinine, eucupine and vuzine are all equally effective in man in 0.5 gm. doses three times daily by mouth (Harvey and Kolb). It is to be recalled that eucupine failed to produce any change in the caprine disease. Both quitenine and quitenidine were ineffective in the human and caprine disorders.[3] Stephens and his associates (1919) have found quitenine of no value in the treatment of human malaria. Giemsa, Weise and Tropp (1926) had the same experience in treating bird malaria, while Dixon and Premankur (1927) found that quitenine was the least toxic to striated muscle of a series of quinine derivatives. Santesson (1892) and Bilsma (1920) have shown that the other drugs mentioned above cause early fatigue of striated muscle. Shaw's work (1928) upon the partition coefficients between red cells

[3] The cinchona derivatives used were supplied by the courtesy of Rare Chemicals, Inc., Nepera Park, N. Y. Dr. W. W. G. MacLachlan of Pittsburgh very kindly supplied the hydroxyethylapocupreine.

and solutions *in vitro* of various compounds related to quinine indicates that oxidation of the vinyl group to the carboxyl, as in quitenine and quitenidine, leads to a complete loss of the ability of the drug to penetrate the cells. Perhaps this change in physico-chemical property is the basis for the ineffectiveness of these drugs in the treatment of myotonus as well as of malaria. No explanation is suggested for the poor response to atebrin.

Certain drugs appeared to accentuate the myotonus. This was more difficult to judge than the relief of the symptom. Most conspicuous in this report was prostigmin, though on the few tests obtained pilocarpin brought about rigidity after a five-minute rest. Prostigmin in the doses used reversed a previous beneficial response from quinine. There was some slight suggestion that ephedrine and barium sulphate and guanidine increased the rigidity but failed to reverse the effect of a previous injection of quinine. In no case were toxic doses of these drugs given. The usual rule was to administer to the adults the maximal safe therapeutic dose for man, to the kids one third or one half of the above. Myotonic patients on quinine therapy studied carefully with dynamograph records before and after administration of barium and guanidine showed no change; the salts of potassium, calcium and magnesium were also without benefit (Harvey and Kolb). The failure to respond to alcohol deserves some mention. Benefit to the human disorder has been observed by Russell and Stedman, and by Kennedy and Wolf. Our own experience with alcohol in myotonic patients has been disappointing.

The evidence presented here clearly supports the impression that the "nervous goats" of Tennessee are suffering from a condition identical with that of myotonia congenita in man. The recognition of a disease condition in animals comparable to one affecting man immediately offers opportunities of study which up till now have not been available for clinical research. The recent advances in the understanding of the divergent conditions, myotonia and myasthenia gravis, and the many questions still unanswered suggest that these animals may prove to be of considerable assistance in furthering these studies.

SUMMARY

1. A description is given of a form of congenital myotonia occurring in goats. The animals suffer from transient attacks of muscular

rigidity brought on by strenuous exertion. Typical seizures, lasting 10 to 20 seconds, occur when the goats are startled and make a sudden effort to leap away. Following a seizure there is a refractory period of 20 to 30 minutes during which the muscular rigidity cannot be induced. The tendency to myotonia is increased in cold weather.

2. Four goats—a billy, a nanny and their two kids—exhibiting this peculiar form of myotonia, were subjected to study. The symptom disappeared in the nanny during pregnancy and could not be induced until one month after its termination. It did not appear in the kids before the age of one month.

3. A typical myotonic reaction could be demonstrated when the muscles were percussed.

4. Galvanic electrical stimulation of the muscles produced simple twitches; painless faradic stimulation produced simple tetanus; painful faradic stimulation produced a prolonged after-contraction.

5. Biopsy studies showed that histologically the muscles were normal.

6. Quinine and other cinchona derivatives, with the exception of quitenine, quitenidine, eucupine and atebrin relieved the myotonic state.

7. Prostigmin and pilocarpine aggravated the symptom and prostigmin reversed the previously beneficial effect of quinine. Although there was some suggestion that ephedrin and barium increased the symptom, they failed to reverse the effect of quinine.

8. Atropine, adrenaline, potassium, calcium, magnesium, guanidine and alcohol failed to modify the myotonus.

9. Attention is directed to the fact that this form of congenital myotonia in goats is practically identical with human myotonia congenita or Thomsen's disease. The animals therefore afford valuable experimental material for a study of the myotonic state.

The author wishes to thank Dr. O. R. Langworthy and Dr. Sam L. Clark for their kind help and many suggestions.

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THE EFFECT OF ALCOHOLIC INTOXICATION AND ETHER ANESTHESIA ON RESISTANCE TO PNEUMOCOCCAL INFECTION

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In reviewing the literature on the effect of alcoholic intoxication on infection and immunity, one is impressed by two facts. First, that while it is rather generally agreed that infected intoxicated animals die sooner than similarly infected non-intoxicated ones, it is fair to say that very few of the studies have been adequately controlled. Second, that no satisfactory explanation has as yet been established for any resistance-lowering effect that alcoholic intoxication may exert. Evaluation of the results in the literature is often made difficult by the fact that comparatively few writers specify the amount of alcohol given, the mode and duration of its administration, and whether or not intoxication was actually established. *In the following review when data such as these are not specified they were lacking in the original papers.* This survey of previous work is presented here because the author has been able to find no review of the subject in the literature.

Koch (1), in 1884, observed that during cholera epidemics most of the people who became sick had been guilty of excesses in the use of alcohol. During his studies on experimental cholera, Koch (2) found that rabbits that were intoxicated by having alcohol introduced into their stomachs, died sooner than similarly infected control animals. Doyen (3), in 1885, confirmed Koch's observations. He believed that the deleterious effect was due to irritation of the gastric mucosa and to some local effect of the alcohol upon the acidity of the stomach. This conjecture, however, was not confirmed by his own control experiments, for the susceptibility to cholera did not rise after the administration into the stomach of croton oil, epsom salts or cantharides.

Thomas (4), in 1893, injected cholera vibrios intravenously into rabbits, some of which received intoxicating doses of alcohol by mouth on two successive days.

He found that all of the intoxicated animals died from the infection, whereas all of those that had not been given alcohol recovered, even though the infecting dose in the controls in each instance was larger than that given the intoxicated rabbits.

The first extensive experiments on the subject in this country were made by Abbott (5) in 1896. Using cultures of diminished virulence, he found that the native resistance of rabbits to infection by the streptococcus pyogenes was markedly diminished through the influence of alcohol when the latter was given daily to the stage of acute intoxication. A similar, though by no means so conspicuous diminution of resistance to the bacillus coli communis occurred in rabbits that were given alcohol. When the staphylococcus pyogenes aureus was used, there was no difference between the resistance of the intoxicated and the control animals. Although Abbott's studies lacked uniformity, it was quite apparent that the susceptibility of rabbits to certain types of infection was increased through the effect of prolonged alcoholic intoxication. With regard to the predisposing influence of alcohol, Abbott believed that it was due to the results of structural alterations consequent upon its direct action on the tissues, although he states that "no such alteration could be found by microscopic examination." No statement is made, however, of what tissues or organs were studied, or whether the sites of infection were examined. But he believed that a closer study of the tissues of these animals would have revealed in them structural changes of such a nature as to indicate disturbances of important vital functions of sufficient gravity to account for the loss of native resistance.

In 1897, Deléarde (6) published experiments on the influence of alcohol on immunization against rabies, tetanus and anthrax. The experiments were conducted in three forms; (1) 10 cc. of 45 per cent alcohol were given daily at the same time as the immunization; (2) during a period of 60 days before the immunization; (3) and after immunity had been attained. He stated that no immunity could be attained when alcohol was given at the same time as the vaccination against tetanus and anthrax, but that immunity which had already been acquired could not be destroyed by alcohol. Alcohol given previous to, but stopped before the beginning of the immunization, did not prevent the development of immunity against rabies.

Laitinen (7), in 1900, studied the effect of alcohol on the resistance of various animals to anthrax, tubercle bacilli, diphtheria and diphtheria toxin. Alcohol was given daily for 61 days before the subcutaneous inoculation of a virulent anthrax culture. The quantity of alcohol used was not less than 5 cc. of 25 per cent ethyl alcohol per kilogram per day; and "in many instances the animals were distinctly intoxicated." The duration of intoxication was, however, not given. The anthrax experiments demonstrated quite unmistakably the deleterious effect of the alcohol. Practically all of the alcoholized animals died of anthrax septicemia, whereas most of those that had not been given alcohol recovered. The survival period of the controls in each instance was longer than that of the animals which had been given alcohol. Labbé (8) found that alcohol injected intraperitoneally in doses sufficient to cause intoxication in guinea pigs did not affect the

course of anthrax or diphtheria infection. Goldberg (9) studied the influence of intoxication upon the native immunity of pigeons to anthrax. Pigeons, which are natively highly resistant to anthrax, were intoxicated at the time of the infection with 1.5 cc. to 3 cc. of 40 per cent brandy, repeated doses being given when necessary to maintain intoxication for several days. For controls he used intoxicated non-infected pigeons. The results are inconsistent and lack uniformity, but there was perhaps a slight tendency to a quicker death in the infected intoxicated animals as compared with the non-infected intoxicated group. Goldberg (10) studied the effect of chronic alcoholism in pigeons that had been given about 1 cc. of 40 per cent brandy for as long as 5 months, and concluded that "chronic alcoholic intoxication in pigeons, as soon as it has given rise to degeneration of internal organs, reduces the natural immunity to anthrax." No mention is made, however, of what organs showed degeneration or of what tissues were studied microscopically, except that one pigeon showed fatty degeneration of the liver.

Laitinen's experiments (7) with tubercle bacilli in rabbits which received 5 cc. of absolute alcohol daily for as long as 34 days after the infection failed to reveal any effect of the alcohol. Further experiments in which rabbits were "scarcely intoxicated," by receiving 5 to 10 cc. of 25 per cent alcohol daily for as long as 60 days after infection with tubercle bacilli, failed to reveal any difference in the alcoholized group as compared with normals. Kern (11) gave guinea pigs "for a long time" daily doses of alcohol ranging from 1 cc. of 10 per cent ethyl alcohol to 5 cc. of 25 per cent ethyl alcohol per kilogram of body weight. The animals were inoculated with 1/50,000 gram of human tubercle bacilli from a glycerin-agar culture. Most of the animals died with intercurrent pneumococcal infection. Of those that survived the intercurrent infection, all died with widespread tuberculosis with no difference between those animals that received alcohol and those that did not receive alcohol. Roos (12) found no increase in the susceptibility of guinea pigs to the tubercle bacillus when given 35 cc. of wine per kilogram daily. No statement is made whether or not the animals were actually intoxicated.

Rubin (13) found that rabbits given a single hypodermic injection of 95 per cent alcohol in quantities sufficient to produce narcosis, and then infected with streptococci and pneumococci, died earlier than controls. Similar results were obtained in rabbits into which ether or chloroform was injected hypodermically and which were then infected with streptococci or pneumococci.

According to an abstract of Ausems' dissertation (14), the original of which was not obtainable, he found that rabbits that received small doses of alcohol daily exhibited a decrease in resistance toward various infectious processes. Small doses of alcohol administered during the course of infection produced a lowering of the body temperature of the experimental animals, although it did not alter or modify the course or the intensity of the disease. Large doses of alcohol, however, produced a marked increase in the duration and intensity of the infection.

Laitinen (7) was unable to demonstrate any increase in susceptibility or diminution of native resistance against diphtheria in experiments in which guinea pigs

were intoxicated by a single administration of 2.5 cc. of absolute alcohol, and then infected with a virulent diphtheria culture.

Hodge (15) stated that distemper occurred more frequently in dogs that were receiving as much as 100 cc. of 33 per cent alcohol daily during a period of 2 years.

Fernandez (16) studied the action of 4 to 5 cc. of 33 per cent alcohol injected intravenously in rabbits in experimental B. typhosus infection, and found little or no difference from similarly infected controls.

Kreparsky (17) studied the healing of wounds and found that alcoholism lessens the number of white cells in the area of repair, and that the repair of wounds takes place more slowly in human alcoholics. He attributed this to an insufficient supply of white cells at the area undergoing repair. Kreparsky's observations were not concerned with infection and were reported in a single paragraph. As we shall show later, the effect of intoxication upon leucocytic emigration exerts a most important influence upon resistance to infection.

Kruschilin (18) studied the effect of alcohol upon the clearing capacity of the blood in rabbits. Two quantities of alcohol were injected intravenously, a large dose-10 cc. of 25 per cent alcohol per kilogram, which produced narcosis for 10 to 15 minutes with complete recovery in 30 to 40 minutes; a small dose of 5 cc., which produced no visible effect on the animal, and an intraperitoneal dose of 10 cc., which produced narcosis in 15 minutes which lasted for about 45 minutes. In most instances, a single injection of alcohol was given several minutes before the bacteria were injected intravenously. Spores of B. subtilis and B. anthrax and a virulent and a non-virulent strain of staphylococcus pyogenes aureus were used as infecting agents. Blood cultures were made at periodic intervals from the median artery of the rabbit's ear. There was no significant difference in the clearing capacity of the blood of the alcoholized rabbits and the controls, when either the spores of B. subtilis or the virulent or avirulent strain of staphylococcus pyogenes aureus were used. When anthrax spores were injected, a prompt and complete clearing of the blood occurred in both the alcoholized group and the controls, but there was a quicker reappearance of the spores in the alcoholized animals than in the non-alcoholized controls. Kruschilin concluded that the smaller doses of alcohol exerted no effect on the phagocytes, but that the larger doses, which produce narcosis for about one-half hour, cause a weakening of phagocytosis and an "alteration of intracellular processes." There was, however, no evidence submitted for his last conclusion.

Numerous investigators have reported that alcohol inhibits phagocytosis *in vitro*. Horsley and Sturge (19) in their book "Alcohol and the Human Body" state: "It is now proved that alcohol, even in tiny doses, paralyzes more or less the white cells, which thus cease from exercising their microbe-destroying function." The literature, however, provides no acceptable evidence for that statement. It is true that many investigators have drawn the same conclusion from their experiments, but there are sources of error in those experiments which really render it unacceptable. To test the phagocytic power of the leucocytes, Rubin (13) used only one single intoxicated rabbit. Each of 2 rabbits was given 3 cc.

of an emulsion of carmine intraperitoneally. One was then narcotized with alcohol hypodermically. Neither the duration of intoxication nor the amount of alcohol was stated, except that the rabbit was given a second smaller dose of alcohol 4 hours after the first dose. After 24 hours the peritoneal exudate was examined. In the normal rabbit, 14 per cent of the leucocytes contained carmine granules as compared with 6 per cent for the rabbit which had received alcohol hypodermically. He concluded from this single animal that alcohol inhibits the phagocytic ability of the leucocytes. No mention was made of any effect of alcohol upon leucocytic emigration. In another publication, Rubin (20) used various bacteria suspended in defibrinated dog blood and found that phagocytosis *in vitro* was inhibited by the addition of alcohol in 1:50 concentration. In a concentration of 1:1,000, phagocytosis was increased. It is needless to draw attention to the fact that alcohol could not reach a concentration of 2 per cent in the most intemperate drinker, since a concentration of alcohol in the blood of more than 0.8 per cent is not compatible with life (21, 22). Stewart's *in vitro* experiments (23) are open to the same objections as those of Rubin (20), in that he used concentrations of alcohol that could not possibly occur in the circulating blood, *e.g.*, 5, 10 and 20 per cent. Even in the last concentration, however, phagocytosis was not completely suspended.

Di Macco (24) found that *in vitro* phagocytosis was impaired by about half after 24 hours when alcohol was added to a leucocyte-typhoid bacillus mixture in 1:1,000 concentration—again a concentration that is never reached in the blood after the ingestion of alcohol. In another publication (25), he found that in acute intoxication in guinea pigs the phagocytic activity was not modified.

To study the action of alcohol upon the phagocytes, Parkinson (26) employed the following method. Blood was dropped into 1.5 per cent sodium citrate and centrifugalized. The leucocytes were then mixed with normal saline and with different strengths of absolute alcohol; 1 in 100, 1 in 50, 1 in 25 and 1 in 5, and these were allowed to stand, with occasional stirring, for about one hour. The corpuscles were then centrifugalized and mixed with human serum and staphylococcus aureus. No appreciable difference in the phagocytic index was found except in the case of the leucocytes that had been exposed to the 1 in 5 concentration. Here the leucocytes were, however, found to be dead, broken and granular in appearance. Experiments were then made to determine at what concentration of alcohol the leucocytes are affected, and for this 2 dilutions of alcohol were used, 1 in 8 and 1 in 10. While normal phagocytosis took place in the latter dilution, phagocytosis in the former was lowered to one-half the normal, and although the corpuscles were not dead, many were markedly damaged in appearance. Parkinson concluded, therefore, that alcohol has no effect upon the phagocytic activity unless present in such concentrations as to seriously injure the vitality of the cells. Moreover, it is impossible for the alcoholic content of the blood of alcoholics to reach a concentration of 12.5 per cent (1 in 8), at which strength it begins to show its effect upon the cells.

Reich (27) found that phagocytosis of tubercle bacilli by human leucocytes in

human serum bore no constant relation to the amount of alcohol consumed. In Hamburger's investigation (28) on the effect of various substances affecting *in vitro* phagocytosis of carbon particles, a statement is made that alcohol increased phagocytosis. No data, however, are given to substantiate this statement. Oker-Bloom (29) could come to no conclusions from his investigation of the influence of alcohol upon phagocytosis.

Graham (30) found that ether anesthesia in human beings and rabbits was followed by decreased phagocytosis of bacteria *in vitro*, but Torraca (31) was unable to confirm this. When ether was added *in vitro* to the phagocytic system, concentrations of 1 to 4,000 or less increased phagocytosis of staphylococci, while Graham found that ether in concentrations of 1 to 5 per cent decreased the phagocytosis of attenuated streptococci.

Franzen (32) studied the effect of immunity reactions *in vitro* and found that up to an alcoholic concentration of 6 per cent, alcohol favored agglutination, showing that the antibody is not destroyed even by this concentration of alcohol. Graham (30) showed that ether in concentrations as high as 10 per cent had no effect on the agglutination of typhoid bacilli in immune agglutinating serum.

Friedberger (33) stated that the single administration of a small quantity of alcohol into the stomach of rabbits which had been previously vaccinated with a killed culture of cholera vibrios caused an increase in the agglutination titer as compared with normals. In 3 animals the administration of alcohol during a period of some weeks before immunization was begun decreased the quantity of antibody produced, but Fraenkel (34) was unable to confirm this. To study the effect of chronic alcoholism upon active immunization, Parkinson (26) injected a staphylococcal vaccine into a rabbit that had been under the influence of chronic alcoholism for 6 days. Whereas a normal rabbit responded with a slight rise in the opsonic index, the intoxicated rabbit's index did not rise above normal. Parkinson (26) stated that the subcutaneous injection of 1 cc. of alcohol in a single rabbit increased the opsonic index against staphylococci within 24 hours, while the single subcutaneous injection of 4 cc. of alcohol in another rabbit lowered the opsonic index temporarily. Abbott and Gildersleeve (35) found that the administration of daily doses of alcohol in one rabbit for as long as 12 days was accompanied by a lowered opsonic index for staphylococci, but that the index quickly returned to normal with the cessation of the alcohol. The amount of alcohol needed to bring out this result had no influence on the resistance of the animal to infection.

Abbott and Bergey (36) found that the serum of rabbits which received less than 150 cc. of absolute alcohol in less than 25 days possessed a lower degree of reactivating power upon a heated hemolytic immune serum than the serum of normal rabbits, while the serum of rabbits which had received more than 150 cc. of absolute alcohol over more than 25 days either showed no appreciable alteration in its complement content, or it possessed even a greater degree of reactivating power than the serum of normal rabbits. Luttinger (37) studied the influence of

alcohol given by mouth, injected subcutaneously and intraperitoneally, on humans, monkeys and guinea pigs and found that the complement was increased.

Abbott and Bergey (36) investigated the influence of alcohol upon the specific reaction of rabbits previously immunized against an alien blood, and found a slight reduction in hemolytic power. They further endeavored to determine what effect alcohol had upon the bactericidal capacity of the blood. Their results, however, were so discordant that no definite conclusions could be formulated, inasmuch as sometimes the serum of an intoxicated rabbit showed less bactericidal activity than the serum of a normal animal, sometimes an equal bactericidal power, and just as frequently even a more pronounced bactericidal action. Laitinen (7) could demonstrate no appreciable difference in the bactericidal capacity of the blood against typhoid bacilli in rabbits that received 0.1 cc. of absolute alcohol per kilogram daily for 18 days.

The temperature in acute intoxication in experimental animals as well as in human patients falls markedly. The fact that alcohol causes a lowering of the temperature of the body is now fairly well established (12, 14). We have found that the same is true in patients in the operating room and also in animals, when either ether or avertin anesthesia is given. Pawlowsky (38) was unable to demonstrate any particular influence of lowering of the body temperature on the resistance of guinea pigs to staphylococcal infection.

Eggers (39) studied the effect of salts normally present in the serum and found that calcium had a stimulating action on the leucocytes. This was confirmed by Hamburger (40), who found that calcium increased the movements of the phagocytes *in vitro*. Cloetta and Thomann (41) noted that under alcohol narcosis the calcium content of the plasma fell 8 to 15 per cent. We have found that the normal calcium content of the blood of rabbits ranges between 14 and 20 mgm. per cent. Deep alcoholic narcosis has never lowered the blood calcium more than 3 mgm. per cent.

Most authors agree that alcoholism is a potent predisposing cause of pneumonia. Osler (42) writing about pneumonia says: "the most potent predisposing factor is perhaps the lowered resistance due to alcohol." In an effort by Osler to determine the mortality rates, it was found that 18.5 per cent of deaths occurred in total abstainers, 25.4 per cent in moderate drinkers, and 52.8 per cent among those known to have been using spirits to excess. Welch (43) stated: "This lowered resistance is manifested both by the increased liability to contract the disease and by the greater severity of the disease." Horsley and Sturge (19) thought that "the predisposition to pneumonia in alcoholics is due to the fact that the repeated taking of alcohol leads to a dilatation of the blood vessels of the lung, and these vessels being extremely numerous, a tendency to congestion occurs. Lungs in this condition of incipient congestion are readily affected by climatic falls of temperature, and by the presence of bacteria, and the outlook when such lungs become diseased is proportionately serious." One of the most convincing pieces of clinical work regarding the alleged effect of alcohol as a predisposing etiological factor in pneumonia was supplied by Capps and Colemann (44). Of

the total number of cases, 3,422, they found that for any particular age period there was a definite increased mortality in proportion to the degree of consumption of alcohol. Thus between 30 and 39 years of age, the mortality of the abstainers was 18.4 per cent, that of the moderate drinkers 29.1 per cent, and of the heavy drinkers 42.5 per cent. And taking the mortality for the whole series of 3,422 cases, excessive drinkers showed a mortality of 49.87 per cent, moderate drinkers 34.4 per cent, and abstainers or occasional drinkers 22.45 per cent.

EXPERIMENTS

One gleans from the numerous papers dealing with the effect of alcohol upon experimental infections that it is rather generally agreed that infected intoxicated animals die sooner than similarly infected non-intoxicated controls. The manner in which alcohol lowers resistance has, however, not been satisfactorily explained. The present experiments were carried out, therefore, in order to determine whether alcoholic intoxication lowers the resistance to pneumococcal infection, and if so, to attempt to discover the mechanism by which it exerts its effect. It was decided at the outset to study the effect of intoxication on well immunized animals in order that any resistance-lowering potentiality that might be present would be put to as severe a test as possible.

In the experiments here reported, 175 rabbits have been used. In every experiment the procedure has been to immunize a group of rabbits by the intravenous injection of Type I antipneumococcus serum, to intoxicate some of them, and then to infect all of them and, in addition, intoxicated and non-intoxicated non-immune controls, with the same dose of Type I pneumococci intradermally.

To produce intoxication 40 cc. of 20 per cent ethyl alcohol was given by stomach tube, smaller doses being repeated when necessary. The animals were kept in a stuporous condition throughout the experiment. Blood cultures were made from all of them at intervals of several hours. The sites of infection were followed macroscopically throughout, and at the termination of the experiment they were removed for microscopic study. In some instances the sites of infection were removed at intervals in order to follow the course of the local infection microscopically.

In a type experiment, 12 rabbits were immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. Sev-

eral hours later, 6 were intoxicated with alcohol and all these, and also intoxicated and non-intoxicated control animals, were infected intracutaneously in the flank with 0.1 cc. of an 8-hour Type I pneumococcus culture. The intoxicated non-immunized rabbits and the non-intoxicated non-immunized rabbits gave a positive blood culture at 4 hours, with 5 to 10 colonies per 10 drops of blood, and death ensued in each instance within 18 hours. The intoxicated immunized rabbits showed a positive blood culture with 2 to 4 colonies at 9 to 10 hours, and death occurred from septicemia within 24 hours. The non-intoxicated immunized rabbits did not develop a positive blood culture, and survived.

The non-intoxicated immunized group developed macroscopically only a minute erythematous lesion. In the non-intoxicated non-immunized rabbits there developed a large, edematous, hyperemic and purpuric lesion which extended to the belly surface. In striking contrast, no macroscopic lesion developed in any of the intoxicated rabbits. Microscopic examination of the sites of infection revealed a dense leucocytic infiltration in all non-intoxicated animals. In the intoxicated rabbits, in sharp contrast, there was practically no leucocytic emigration, and bacteria were soon present in swarms in the tissues in the immunized as well as in the non-immunized group. In the non-intoxicated immunized animals there was an abundant leucocytic exudate and no bacteria could be found in the sections after 9 hours (See figures 1 to 5).

In some instances rabbits were first intoxicated and then immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. The results in these animals were identical with those described above in which the serum was given several hours before intoxication was established.

Experiments of the same type carried out on immunized rabbits kept under ether or avertin anesthesia gave precisely the same results as those described above in the case of the intoxicated immunized animals.

Since the temperatures of the intoxicated rabbits fall from the normal level of 102°F. to a level as low as 97°F., an experiment was carried out to control this factor. 18 rabbits were highly immunized by the intravenous injection of 10 cc. of Type I antipneumococcus

serum. After several hours 6 were then intoxicated with alcohol, and 6 others were strapped flatly on their backs. This position lowers the temperature of a rabbit markedly. When the temperature level had reached that of the intoxicated animals, these animals together with the intoxicated animals, and the non-intoxicated immunized and non-immunized controls, were infected intracutaneously with the usual dose of pneumococci. The temperatures of the inverted rabbits remained at a low level throughout the experiment, never rising above 99°F. The results were identical with those recorded above. The leucocytic emigration in the intoxicated animals was negligible. In contrast, there was a marked local outpouring of leucocytes in all of the non-intoxicated immunized rabbits, and lowering of the temperature did not interfere with the emigration. Myriads of pneumocci were found at the infected sites in the intoxicated animals and these developed a septicemia and died, whereas no bacteria could be found in the lesions of any of the immunized non-intoxicated animals after 9 hours, regardless of their temperature, and they all survived the infection.

To determine whether a similar result would occur in the lung, 4 rabbits were immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. After several hours, 2 were intoxicated with alcohol in the usual manner. The 4 rabbits together with 2 normal control rabbits were given intra-tracheally 0.2 cc. of a pneumococcus culture diluted with broth to a volume of 2 cc. The intoxicated non-immunized and the normal control rabbits gave a positive blood culture within 5 hours with an average of 250 colonies per 10 drops of blood, and death occurred within 12 hours. The intoxicated rabbits which received serum did not develop a septicemia until 18 hours, at which time the plates averaged 180 colonies per 10 drops of blood, and they died at 22 and 24 hours, respectively. The non-intoxicated rabbits which received serum did not develop a septicemia and were in excellent condition when they were killed at 22 and 24 hours for comparison of their lesions with those of the other rabbits. Microscopically, as was seen in the skin, there was an abundant leucocytic exudate in the lungs of the immunized non-intoxicated animals, and not a single bacterium could be found, while in the intoxicated animals which received serum there was only a slight leucocytic

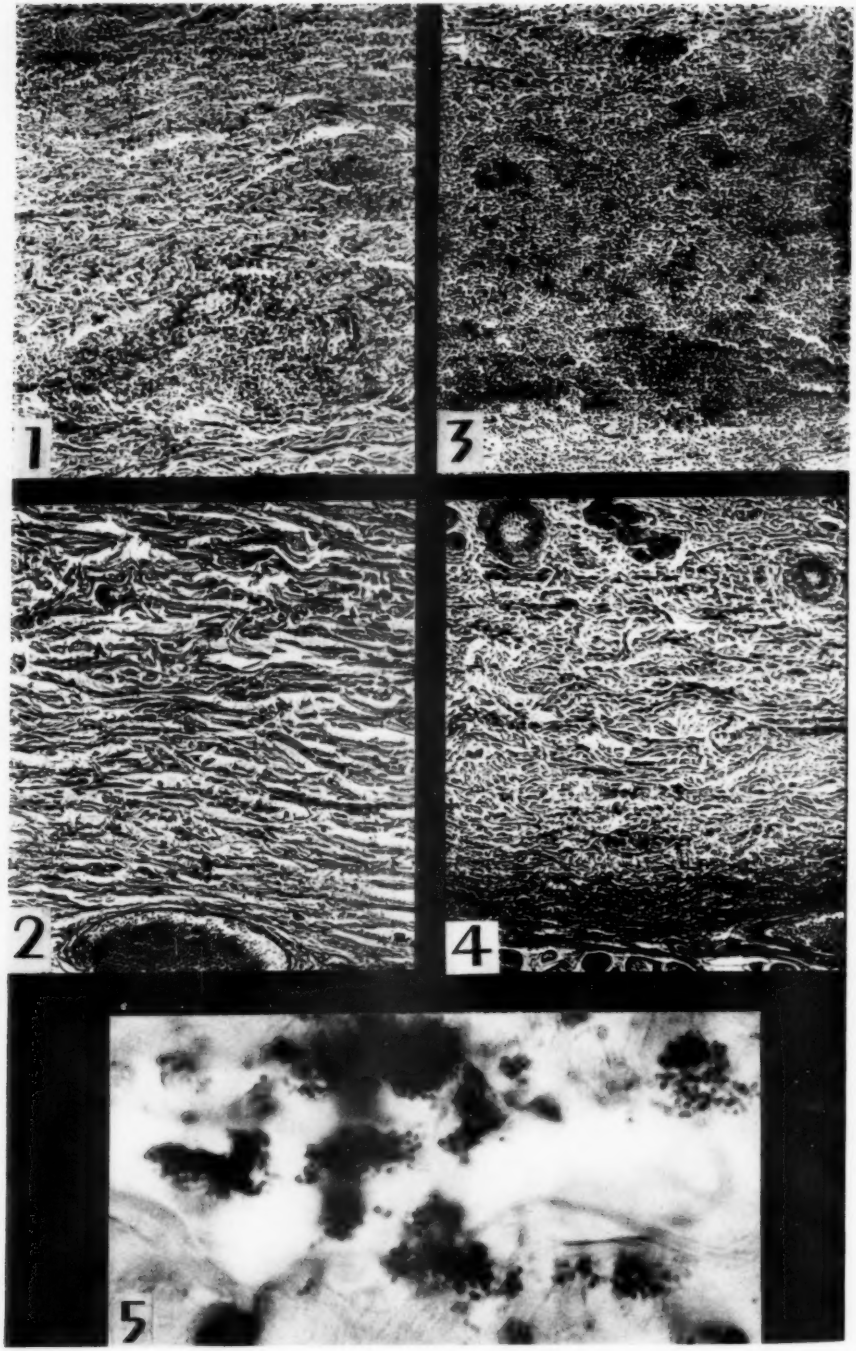

FIG. 1–5

emigration and there were myriads of pneumococci, forming large colonies in the alveoli and smaller bronchi (See figs. 6 and 7). Although there was a dense leucocytic exudate in the non-intoxicated non-immunized rabbits, there was a diffuse growth of pneumococci throughout the lung.

Blood counts showed that the scarcity of leucocytes at the infected sites in the intoxicated animals in these various experiments could not be attributed to the development of leucopenia, for that did not occur.

In order to determine whether intoxication would inhibit leucocytic emigration in the skin when another irritant was injected intradermally, 2 rabbits were used. One was intoxicated in the usual manner and maintained in a stuporous condition throughout the course of the experiment. Both rabbits were then injected intradermally with 0.1 cc. of an aleuronat suspension in each flank. The inoculation sites were observed macroscopically, and at the end of 5 hours and 10 hours, respectively, they were removed for microscopic study. The non-intoxicated rabbit developed a large erythematous lesion, in contast to the intoxicated rabbit which failed to develop any macro-

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FIG. 1. SITE OF INFECTION FROM A 6-HOUR IMMUNIZED, NON-INTOXICATED RABBIT, SHOWING THE EARLY INFLAMMATORY REACTION WITH VIRTUALLY ALL OF THE BACTERIA PHAGOCYTIZED

FIG. 2. SITE OF INFECTION FROM A 6-HOUR IMMUNIZED, INTOXICATED RABBIT, SHOWING ALMOST A COMPLETE ABSENCE OF LEUCOCYTES

The bacteria are agglutinated in clumps, which may be seen as black dots, particularly just above the large vessel (see fig. 5).

FIG. 3. SITE OF INFECTION FROM A 12-HOUR IMMUNIZED, NON-INTOXICATED RABBIT, SHOWING AN ABUNDANT INFLAMMATORY RESPONSE

All of the bacteria have been phagocytized

FIG. 4. SITE OF INFECTION FROM A 12-HOUR IMMUNIZED, INTOXICATED RABBIT

The dark zone at the bottom of the corium represents masses of pneumococci which have proliferated, uninhibited by the antibody (see fig. 5 for agglutinating action of antibody). Note that in spite of the great numbers of bacteria there is no leucocytic emigration.

FIG. 5. OIL-IMMERSION FIELD FROM A 6-HOUR IMMUNIZED INTOXICATED RABBIT, SHOWING THAT THE CLUMPS OF AGGLUTINATED BACTERIA GROW FREELY WITH NO LEUCOCYTES TO COMBAT THEM

scopic lesion whatever. Sections directly through the injected aleuronat revealed a moderately dense leucocytic infiltration in the normal rabbit, in sharp contrast to the intoxicated rabbit in which there was practically no leucocytic emigration even after 10 hours.

The influence of alcoholic intoxication upon the phagocytic activity of the leucocytes was therefore investigated. In the first experiment, 4 rabbits were immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. After several hours, 3 were intoxicated in the usual manner and intoxication was maintained throughout the course of the experiment. 5 cc. of an aleuronat suspension were then injected into each pleural cavity, with a view toward inciting an outpouring of alcohol-containing exudate from the blood into the pleural cavity. It was hoped that at least a number of leucocytes sufficient for a study of *in vivo* phagocytosis would escape into the cavity. Of a concentrated culture of virulent Type I pneumococci 2 cc. were injected into the pleural cavity at 6, 10 and 14 hours after injection of the aleuronat. At 45 minutes later the animals were killed and smears were made from all parts of the pleural cavities. Although there was a dense leucocytic exudate in the control animal and abundant phagocytosis, the thin pleural exudates of the intoxicated animals were practically devoid of leucocytes. Only rarely could a single leucocyte be found in a stained smear. The bacteria, however, were found in large clumps, agglutinated under

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FIG. 6. FROM THE LUNG OF AN IMMUNIZED NON-INTOXICATED RABBIT 22 HOURS AFTER INFECTION, SHOWING THE DIFFUSE PNEUMONIA

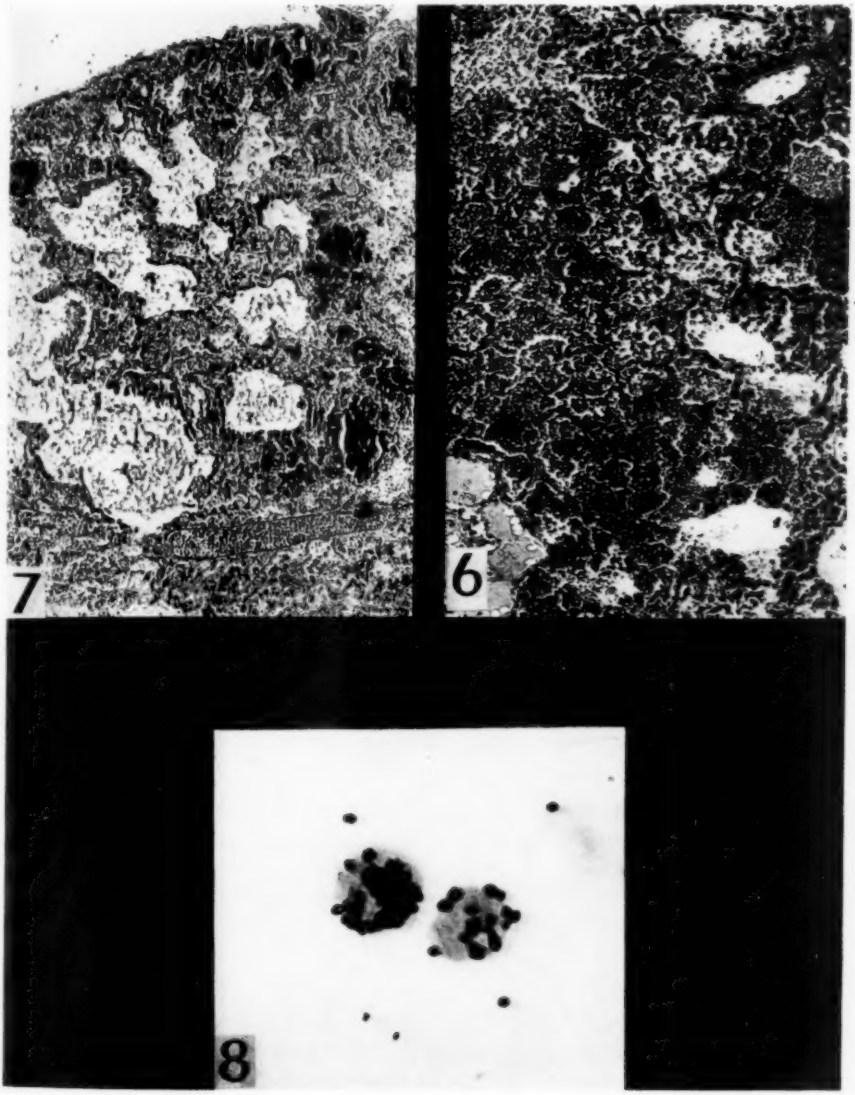
All of the bacteria have been phagocytized and destroyed.

FIG. 7. FROM THE LUNG OF AN IMMUNIZED INTOXICATED RABBIT 22 HOURS AFTER INFECTION

The black masses are colonies of pneumococci. There is hemorrhage at this stage and some outpouring of fluid, but leucocytes are scanty in comparison with Fig. 6.

FIG. 8. SHOWS ABUNDANT PHAGOCYTOSIS OF AVIRULENT PNEUMOCOCCI BY LEUCOCYTES FROM THE PLEURAL EXUDATE FROM AN INTOXICATED RABBIT

The leucocytes had been made to emigrate before intoxication was induced. The alcoholic content of the pleural exudate was above 600 mgm. per cent. No difference could be ascertained between the degree of phagocytosis in intoxicated and non-intoxicated animals under these conditions.



FIGS. 6–8

the influence of the antibody. The analyses of the blood and pleural exudates by the method of Friedemann and Klaas (45) at this time showed an alcoholic content of between 550 and 700 mgm. per cent.

Since the leucocytes failed to emigrate in the intoxicated animals, it was decided to bring them out into the pleural cavity before intoxication was induced, and in order to avoid the agglutination of the bacteria it was decided to use an organism that is phagocytized without the aid of immune serum. In this experiment 24 rabbits were used. 5 cc. of aleuronat suspension were injected into the pleural cavity and 18 hours later, when there was an abundant exudate rich in leucocytes, half of the rabbits were intoxicated in the usual manner. After profound intoxication had been maintained for 2 hours, 2 cc. of a concentrated culture of a readily phagocytizable, non-encapsulated, rough, avirulent pneumococci were injected into the pleural cavity. Stained smears of the pleural exudates made at 45 minutes showed abundant phagocytosis. Virtually all of the leucocytes were engorged with pneumococci (fig. 8). No difference could be made out between the number of leucocytes containing bacteria or the average number of bacteria ingested, in the intoxicated as compared with the control animals. Analyses of the blood and pleural exudates at this time showed an alcoholic content of about 650 mgm. per cent, a concentration even higher than is encountered in the blood in intoxicated human beings (21, 22, 46). The alcoholic content of the blood in these animals was never more than 40 mgm. higher than that of the pleural exudate. Since phagocytosis was very active in these exudates, no attempt was made to perform any *in vitro* tests. This experiment demonstrated that the effect of intoxication in destroying immunity is not a result of a paralysis of the phagocytic power of the leucocytes. In the deeply intoxicated animal they are able to phagocytize bacteria avidly if they come in contact with them.

To ascertain whether phagocytosis in the skin was impaired during intoxication 4 rabbits were used. 0.5 cc. of broth, concentrated to ten times its original concentration, was injected intracutaneously into each rabbit's flank in order to call forth a leucocytic exudate. The area infiltrated by the broth was carefully marked. Each animal was then immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. After 5 hours, 2 of the rabbits were in-

toxicated in the usual manner, and after stupor had been maintained for at least 2 hours, 0.1 cc. of a virulent Type I pneumococcus culture was injected intradermally into the center of the injection site. The sites were removed for section after one and one-half hours. Although phagocytosis is not as readily discernible in sections of skin as it is in stained smears from pleural exudates, no difference in the number or degree of phagocytizing leucocytes could be demonstrated.

To determine whether the activity of the cells of the reticulo-endothelial system were impaired during intoxication, 8 rabbits were used. Each was immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. After 2 hours, alcohol was given to half of them in the usual manner. After intoxication was well established, all the rabbits received an additional 5 cc. of antipneumococcus serum intravenously. Ten minutes later, each received 5 cc. of a concentrated Type I pneumococcus culture injected into the portal vein or a mesenteric vein near it. After 5 minutes, the rabbits were killed and their livers removed for microscopic study. Bacterial stains of the sections revealed no detectable difference in the degree of phagocytosis by the Kupfer cells in the intoxicated as compared with the non-intoxicated controls.

To substantiate these findings still further, 3 intoxicated rabbits and 3 normal rabbits were injected with a concentrated culture of rough, avirulent pneumococci into the portal vein or a mesenteric vein near it. After 5 minutes, the rabbits were killed and their livers removed for section. Here, likewise, the stained sections revealed no detectable difference in the degree of phagocytosis by the Kupfer cells in the intoxicated and non-intoxicated animals.

Since these various experiments showed that alcoholic intoxication does not impair the phagocytic power of the leucocytes *in vivo*, a study was made of the effect of intoxication upon the motility of the leucocytes. For this purpose blood was taken both from patients and rabbits in alcoholic stupor, and vital preparations were studied in the warm chamber. Differential counts revealed no difference in the number of non-motile leucocytes in the blood of the intoxicated subjects as compared with that of normal controls. Similar results were obtained in both patients and rabbits under ether or avertin anesthesia.

To determine whether alcoholic intoxication interfered with the clearing capacity of the blood, 18 rabbits were used, 14 of which were immunized by the intravenous injection of 10 cc. of Type I antipneumococcus serum. Of the latter, 8 were then intoxicated with alcohol in the usual manner and were maintained in a stuporous condition throughout the course of the experiment. These, and in addition 2 intoxicated rabbits which did not receive serum, and 2 normal control rabbits, were given 0.1 cc. of a 1:10 dilution of Type I pneumococcus culture intravenously. Blood cultures were made every 5 minutes during the first hour and every 2 hours thereafter for 36 hours. In all of the immunized animals, whether intoxicated or not, the blood became sterile within 30 minutes and remained sterile thereafter and the animals survived. In the non-immune animals, both intoxicated and non-intoxicated, the number of colonies increased progressively, and death followed in each instance within 12 hours.

To determine whether a shorter period of narcosis or anesthesia would have a deleterious effect, 15 rabbits were used. In a type experiment, 3 rabbits were highly immunized by the intravenous injection of 8 cc. of a concentrated Type I antipneumococcus serum. After several hours one immunized rabbit and one control rabbit were intoxicated in the usual manner. After stupor had been attained, the alcohol was discontinued and both rabbits were infected intracutaneously in the flank with 0.1 cc. of an 8-hour virulent pneumococcus culture. The rabbits were not disturbed thereafter. One immunized rabbit under ether anesthesia was similarly infected. The anesthesia, however, was continued for 3 additional hours after the rabbit was inoculated. A non-immunized non-narcotized control rabbit was infected in a similar manner. The normal control rabbit developed a progressive edematous and hemorrhagic lesion which spread rapidly to the belly surface, and it died at 22 hours with an overwhelming septicemia. Neither the intoxicated rabbits nor the anesthetized rabbit developed any lesion whatever at the site of infection until they began to recover from the narcosis, when there appeared a progressive edematous and hemorrhagic lesion which soon spread to the belly surface. The anesthetized rabbits recovered consciousness very rapidly, while the intoxicated rabbits did not begin to do so for

about 5 hours. The intoxicated non-immune rabbit died from an overwhelming septicemia at 20 hours, while the intoxicated immunized rabbit died at 30 hours. The immunized rabbit that was anesthetized with ether for 3 hours after being infected died from septicemia at 40 hours. The immunized non-narcotized rabbit developed only a minute erythematous lesion at the site of infection, and it recovered. This experiment demonstrated that if bacteria can grow uninhibited by the defenses of the body for even 3 to 5 hours, inflammation developing after that may be powerless to overcome them.

DISCUSSION

These experiments make it clear that the loss of immunity during alcoholic intoxication or ether or avertin anesthesia is not due to the fact that the leucocytes are paralyzed, but rather to their failure to emigrate. Since the motility of the leucocytes is not affected, the failure to emigrate appears to be due rather to an effect of intoxication or anesthesia upon the vascular inflammatory mechanism. It will be remembered that the sites of infection in the intoxicated animals did not become erythematous or edematous, even when the bacteria had proliferated to enormous numbers at the site. The same was true when a non-bacterial inflammatory irritant (aleuronat) was used. This appears to be clear evidence that in the intoxicated body the capillaries fail to respond to the presence of an inflammatory irritant with dilatation and increase in their permeability. *In vivo* studies of inflammation under the microscope by previous investigators have shown that when the capillaries react to an inflammatory incitant, not only do they dilate, but changes occur at the same time in the rate of flow of blood through them and, what is important for the present consideration, there are marked alterations in the distribution of the blood cells. The red cells tend to occupy a position as a core in the center of the stream, while the leucocytes are segregated laterally along the capillary wall. In this manner the leucocytes are brought into position for emigration, and they are seen to become adherent to the endothelium and then to escape through the capillary wall into the surrounding tissue. In the absence of capillary dilatation and of the attendant change in blood flow, the margination of the leucocytes does not occur. Since intoxication prevents these

primary changes in the capillaries, the leucocytic margination and adherence to the capillary wall, which appear to be necessary precursors of leucocytic emigration, do not occur. In the sections from the sites of infection in the intoxicated animals one fails to find the adherence of leucocytes along the walls of the capillaries that is so prominent at the infected sites in the control animals. While this may not represent the entire reason for the absence of emigration of the leucocytes in the intoxicated animals, the failure of the capillaries to react to irritants in the usual manner in these animals is the only reason for the absence of leucocytic emigration that has been disclosed by the present studies. In what manner intoxication acts to prevent the usual inflammatory alterations in the capillaries is at present obscure, but that it does so is clearly evident.

These experiments serve to emphasize again the essential rôle of the phagocyte in protection against pyogenic bacteria even when humoral immunity is present in high degree. Rich and McKee (47) have shown that no matter how high a degree of active or passive immunity might be present, pneumococci would grow freely in the tissues of animals deprived of leucocytes by the action of benzol. The histological appearance of the sites of infection in those experiments was identical with that in the present experiments, in that pneumococci proliferated to enormous numbers in the absence of leucocytes at the site. In both instances agglutination of the bacteria in the tissues under the influence of the antibody was evident. In the former case, the pneumococci continued to grow because the body did not contain a sufficient number of leucocytes. In the present case, although abundant leucocytes were present in the blood stream, their failure to escape permitted the progressive growth of the bacteria even in the presence of abundant antibody.

These studies demonstrate that if bacteria are aspirated into the lungs during deep alcoholic intoxication, or during ether or avertin anesthesia, they will grow uninhibited by the defenses of the body during the entire period of unconsciousness, and this, regardless of the amount of immunity possessed by the body against the bacteria. If the bacteria are able to grow unrestrictedly during several hours, they may easily become so numerous that inflammation developing after recovery of consciousness may be unable to overcome them. It

seems not unlikely that these studies may throw some light on the mechanism of the development of pneumonia occurring in intoxicated persons and following prolonged anesthesia. We are interested in learning whether the inflammatory response is also inactive during the stupor produced by other anesthetics and sedatives, and during unconsciousness that is not due to the action of drugs or anesthetics, and we hope to report later on this question.

CONCLUSION

Alcoholic intoxication maintained at the point of stupor destroys resistance to pneumococcal infection in the rabbit. Even in animals rendered highly immune by the intravenous injection of antipneumococcus serum, intoxication deprives them of their immunity. The loss of resistance to the infection appears to be due to the fact that intoxication profoundly inhibits the vascular inflammatory response as long as intoxication is maintained, and in the absence of capillary dilatation and of margination of the leucocytes, leucocytic emigration at the site of infection is negligible and the bacteria, therefore, proliferate uninterruptedly. Similar experiments show that ether or avertin anesthesia has as marked an inhibitory effect on the inflammatory response as has alcoholic intoxication, and produces as marked a loss of resistance to infection.

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PARTIAL ATRESIA OF THE MAIN BRANCHES OF THE PULMONARY ARTERY OCCURRING IN INFANCY AND ACCOMPANIED BY CALCIFICATION OF THE PULMONARY ARTERY AND AORTA

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Atresia or stenosis of the pulmonary artery is frequently encountered in cases of congenital malformation of the heart, in combination with stenosis of the pulmonary valve and other cardiac anomalies. On the other hand, stenosis or atresia of a *branch* of the pulmonary artery is such a rare defect that Bredt (1) in reviewing the possible anomalies of the pulmonary artery agreed with Mönckelberg (2) that stenosis of the pulmonary artery did not occur independently of stenosis of the pulmonary orifice. The following case is reported in detail because there was stenosis of both of the main branches of the pulmonary artery with no stenosis of the pulmonary valves or orifice. This case was, furthermore, most unusual in that the anomaly was associated with medial sclerosis and calcification not only of the narrow portion of the pulmonary artery but also of the main pulmonary trunk and of the aorta. The stenosis of the pulmonary vessels was so marked that it produced right-sided cardiac enlargement and eventually resulted in cardiac failure.

Müller (3), in 1927, reported a case of atresia, or rather agenesis, of the right pulmonary artery combined with coarctation of the aorta and anomalies of the valves of the heart and the lobes of the lungs. This occurred in a 35-year-old woman, dying of puerperal sepsis. No remnant of the right pulmonary artery could be found, nor was there any indication of where it should have arisen. The left pulmonary artery was quite normal. The ductus arteriosus could still be identified but contained only a pinpoint lumen. In his survey of the literature for similar cases, Müller found that both stenosis and

atresia of the pulmonary arteries were extremely rare. Indeed, he could find only four examples and since then, so far as we have been able to determine, no additional cases have been reported.

The essential features of these four cases were as follows: Kessler's case, described by Fürst (4), was that of a girl who died after months of asphyxia and cyanosis. The left pulmonary artery was 4 mm. smaller in diameter than the right. The ductus arteriosus contained only a pinpoint lumen. No other details of the case were given.

In Maugars' case (5), there was narrowing of the left pulmonary artery. A compensatory collateral circulation to the left lung arose from a branch of the abdominal aorta.

Laval's case (6) was that of an 8-month-old child in whom he observed an abnormal origin rather than absence of the left pulmonary artery. The main pulmonary artery did not divide as usual; instead, the artery passed directly to the hilus of the right lung where it gave off a branch. This branch crossed over the right bronchus behind the trachea to the hilus of the left lung, into which it passed in the normal fashion.

Finally, Doering's case (7) was that of a 9-months-old child with complete absence of the right pulmonary artery. The compensatory blood supply to the right lung was from a branch of the innominate artery. The ductus arteriosus was patent and there was hypertrophy of the right side of the heart.

In none of these cases was there any mention of arterial calcification. In fact, only 5 cases of abnormal thickening of the pulmonary artery occurring in infancy could be found in the literature, and in only 3 of these cases was there calcification of the artery.

The earliest case found was that of Durante (8). This report concerned a 7-months-old child who died of peritonitis following an umbilical infection. At autopsy, although the heart appeared normal, the entire pulmonary artery was altered by patches of increased density and plaques resembling those seen in a senile aorta with advanced sclerosis. The intimal lining was smooth. Microscopically, the pulmonary artery showed fatty degeneration and calcareous infiltration in its media, with only slight intimal thickening. The aorta, which grossly seemed normal except for a slight loss of elasticity, showed a lesser degree of the same medial change. No pictures of

this lesion were given. No other abnormality was noted. The author considered this to be a congenital lesion.

Jaffe's case (9) was that of a 2-day-old baby whose mother had had a severe grade of hydramnios. The baby died from a birth injury—a tentorial tear with cerebral haemorrhage. At autopsy the heart was slightly enlarged; the right auricle and ventricle were dilated and the left ventricle somewhat thickened. The foramen ovale was widely open. The gross appearance of the aorta was normal. Rather large patches of intimal fat were noted in the pulmonary artery. Microscopically, throughout all portions of the media of the pulmonary artery there were oval and streak-like necroses which stained blue in haematoxylin and eosin preparations. Many of the necrotic foci were calcified. It was thought that the destruction involved mainly the muscle fibers of the inner portions of the media. Elastic stains showed breaks in poorly stained elastic fibers. The peripheral fibers about the areas of necrosis were well stained, but short and straight, having lost their spiral form. No description of the lungs or pulmonary arterioles was given. The kidneys were described as normal. Jaffe believed that this type of medial necrosis resembled that produced experimentally by Fischer (10) in animals with adrenaline poisoning, and suggested the possibility that some toxic factor of maternal origin might have caused both the fetal arterial necrosis and the maternal hydramnios. The etiology of this case, however, was quite obscure.

Surbek's case (11) was that of a 3-day-old infant dying as a result of a diplococcus infection acquired *in utero*. The pathological change in the arteries was similar to that in Jaffe's case, although it extended throughout the entire arterial system. Surbek emphasized the similarity between this lesion and the "adrenalin-sclerosis" of Fischer. At the time of Surbek's report the syndrome of hyperparathyroidism had not yet gained recognition and the parathyroids were not described. In view of the fact that there was widespread pathological calcification combined with severe chronic diffuse nephritis, the possibility of hyperparathyroidism would certainly not be overlooked today. In addition to the nephritis, and calcification in the kidneys, ovaries and adrenals, there was the most extraordinary medial calcification in the aorta and all its branches and also in the arteries of

the intestines and extremities, the coronary arteries and the pulmonary arteries and arterioles. In the aorta the calcification occurred in streaks in the media, and breaks in the elastica were filled with granulation tissue often containing foreign-body giant cells. The adventitia was greatly thickened and infiltrated with round cells and there was compensatory thickening of the intima. The pulmonary arteries showed the streaks of medial calcification, but only occasionally was there slight intimal thickening and fatty infiltration. The arterioles showed calcification of the inner zone of media with a normal intima. Cellular adventitial infiltration was explained on the basis of the diplococcus infection. Pictures published in this report clearly illustrate the microscopic changes. The lines of ossification of the bones were normal.

Wätjen's case (13) showed some medial change combined with a greater amount of intimal thickening of the pulmonary arterioles, but no calcification. This occurred in a 6 months old girl with a congenital malformation of the heart. There was a transposition of the great vessels, a patent foramen ovale and a defect in the ventricular septum. The left ventricle was twice as large as the right, and the pulmonary artery, which arose from the left ventricle, was thicker than the aorta. The larger branches of the pulmonary artery were widened. Microscopically, the pulmonary arterioles were found to be narrowed by connective-tissue thickening of the intima and by fatty infiltration of the intima and media. A picture clearly shows this change. Wätjen considered the possibility that the arterial process was a "functional-mechanical" alteration resulting from a disturbance in blood pressure brought about by the congenitally malformed heart.

Another case reported by Wätjen (14) and also by Linden (15), although a case of pulmonary artery sclerosis, had as its chief abnormality an obliterative endarteritis. This was the case of an 11 months old child with tremendous dilatation of the right side of the heart and of the pulmonary arteries. There were seven malformed pulmonary veins which emptied into the left auricle. The ductus arteriosus and foramen ovale were both patent. Microscopically, the lumina of the terminal pulmonary arterioles were occluded by intimal proliferation. The larger arterioles showed only compensatory hypertrophy of their medial elastica and muscularis, and dilatation of

their lumina. This arterial change was considered to be a congenital malformation associated with the other congenital abnormalities.

In order to obtain additional information concerning the association between stenosis of the pulmonary artery and arterial calcification in infancy, reports of the first 15,000 autopsies of this hospital were searched with these two conditions in mind. In none of the infants having congenital malformations of the heart or great vessels was arterial calcification noted. Three cases were found, however, in which arterial calcification had been observed in infants whose hearts showed no congenital defect. Abstracts of these autopsy protocols follow:

Autopsy No. 8583 was performed upon a white female infant aged 6 months, who had always been sickly. She died suddenly without obvious cause. At autopsy the heart, particularly the left ventricle, was found to be hypertrophied; the lumina of the coronary arteries were narrowed and showed gross calcification. The aorta seemed elastic but there were transverse gray stripes in the intima of the thoracic portion. Elsewhere the intima was smooth. The renal arteries as they entered the kidneys seemed thickened, with pin-point lumina. The other arteries all appeared normal. Microscopically, there was a remarkable widespread calcification of the small arteries all over the body while the aorta, even in the thoracic portion, showed only slight intimal thickening. The calcification in the small arteries occurred in a ring in the region of the internal elastic lamella. In more advanced stages, fragments of calcium were embedded in a proliferative granulation tissue containing numerous foreign-body giant cells. The narrowed coronary arteries caused scarring of the myocardium, and as a result of the renal arteriosclerosis there was beginning scarring of the kidneys. The bones were quite normal. The parathyroids were small and showed no microscopic lesion.

The lesions in this case closely resembled those described by Surbek. The etiology of the calcification was entirely obscure; it was, undoubtedly, the cause of both the renal and the myocardial changes.

Autopsy No. 11723 was performed upon a 4 months old white female, who apparently had died of cardiac failure after being ill only 24 hours. The heart weighing 50 grams, was slightly enlarged, with a predominant hypertrophy of the left ventricle. The latter was also dilated and its wall showed gross myocardial scars. The aorta appeared normal and the kidneys showed no gross change. The autopsy was limited to an abdominal incision so that the neck organs and parathyroids were not examined. Microscopically, the larger arteries and those of moderate size all over the body showed a lesion which began as calcification of the internal elastic lamella. These areas of calcification became larger until in places they extended through the entire arterial wall. In places the intima was

very thick and the endothelium was lifted up over a loose connective tissue which often contained calcium and foreign-body giant cells. This lesion was identical with that in Autopsy No. 8583 and also with that shown in the illustrations accompanying Surbek's report. In this case, too, the bones were normal.

Again, the etiology of the calcification was quite obscure; but as a result of the coronary arteriosclerosis there was widespread myocardial scarring. In addition, the narrowing of the lumina of the renal vessels by extreme calcification caused an extraordinary nephritis in which there was widespread calcification of the tubules and even of the coagulum usually responsible for crescent formation.

In *Autopsy No. 11704* the distribution of the calcification was similar to that observed in the case reported below. There was, however, no malformation of the arteries or heart. This 11-day-old female infant had an ascending urinary tract infection secondary to a congenital malformation of the genitalia. There was an imperforate anus and vagina, and a bicornute uterus; the heart was normal except for a patent foramen ovale and a partially occluded ductus arteriosus. The aorta showed an intimal streak on its anterior surface made up of a series of little white elevations. Microscopically, the pulmonary arteries and aorta showed a curious calcification of the elastic fibers. In later stages of the lesion the fibers were broken and there were more extensive calcium deposits in the media. There was no cellular reaction or intimal change. Ossification of the ribs was irregular. Tongues of cartilage matrix surrounded by narrow calcified zones extended toward the marrow. The parathyroids were small and not abnormal. The kidneys contained large abscesses which evidently had resulted from an ascending infection, following a cystitis and a ureteritis.

Although each of these cases showed abnormal arterial calcification, they were totally different from the following case:

CASE REPORT

F. S. (H. L. H. No. A-361) was a white male infant who was brought to the Harriet Lane Home at 17 months of age because of cyanosis and cardiac failure.

Family history non-contributory, with the possible exception that the mother had suffered from pernicious vomiting during seven months of this pregnancy. There was no history of syphilis. The blood Wassermann reactions of both parents were negative.

Past history: Birth normal; weight 8 pounds. No cyanosis noted at birth. The mother had noticed that the respirations had always been rapid and that, when cold, the baby became intensely cyanotic. He was breast fed for two months and then given a cow's milk formula. At three months of age orange juice and 10 drops of viosterol daily were added. By one year of age he was receiving a regular infant diet. Four days before admission the infant developed a head cold and the hands and feet became swollen.

Physical examination showed the following positive findings: The infant was remarkably well nourished and well developed. The respirations were rapid. There was intense cyanosis of the hands, feet and lips. The chest bulged on the left side. The heart was markedly enlarged; the rate was rapid. There was a soft systolic murmur and a gallop rhythm. The blood pressure was 98/78 in both arms and in the legs. The lungs were clear. The liver extended to the umbilicus. The hands and feet were cyanotic and oedematous.

Laboratory examinations revealed the following: Red blood cells 7,200,000; hemoglobin 90%; white blood cells 12,500. The urine showed a specific gravity of 1.020 and a moderate trace of albumin; microscopic examination of the sediment was negative. The teleoroentgenogram showed that the heart extended 8 cm. to the left and 2.4 cm. to the right of the mid-line; the total transverse diameter of the chest was 15 cm. Fluoroscopic examination showed that the enlargement of the heart was mainly due to enlargement of the right ventricle. The electrocardiogram showed an inverted T-wave in Leads II and III, and a right axis deviation. Wassermann reaction of the blood was negative. The blood nonprotein nitrogen was 82 mg. per 100 cc. The blood cholesterol was 62 mg. per 100 cc. The total plasma proteins were 4.4 gm. per 100 cc. The albumin-globulin ratio was 1:3.

The *course in the hospital* was progressively downhill. The dyspnea, cyanosis and oedema increased. Ten days after admission the baby died.

Clinical impression: Congenital malformation of the heart belonging to the cyanotic group.

Autopsy No. 15130 was performed five hours after death. It was restricted to an examination of the viscera of the chest and abdomen.

The *final anatomical diagnosis* was as follows: Partial atresia of right pulmonary artery and lower branch of left. Diffuse medial necrosis and calcification, with defect in the elastica of the aorta and pulmonary arteries. Adventitial thickening of pulmonary arterioles. Great hypertrophy of right auricle and ventricle; slight hypertrophy of left ventricle. Patent foramen ovale. Slightly open ductus arteriosus. Mural thrombi in right ventricle. Clubbing of fingers. Chronic passive congestion of the liver and spleen. Acute bronchitis.

Only the pertinent findings are described below.

The *body* was that of a well nourished white baby boy with no visible edema. There was recognizable cyanosis of the fingers and about the lips, which contrasted sharply with the pallor of the remainder of the skin. There was conspicuous clubbing of the fingertips.

Serous cavities: There was no abnormal accumulation of fluid. The surfaces of peritoneum, pericardium and pleurae appeared normal.

Heart: It weighed 150 grams and was tremendously enlarged (fig. 1). The wall of the right auricle was thickened but its lining was smooth. Both venae cavae were normal. The foramen ovale was covered by its usual septum but it was definitely patent and communicated with the left auricle. The tricuspid

valve was perhaps slightly shortened, and thickened along its margin. Its chordae tendineae seemed short compared with the size of the heart. The right ventricle was enormously hypertrophied and widely dilated. The wall measured 12 mm. in diameter. It was thinner at the apex and here there were several gray thrombi adherent to the trabeculae. No defect was found in the ventricular septum. The pulmonary orifice measured 4 cm. in circumference and appeared quite nor-

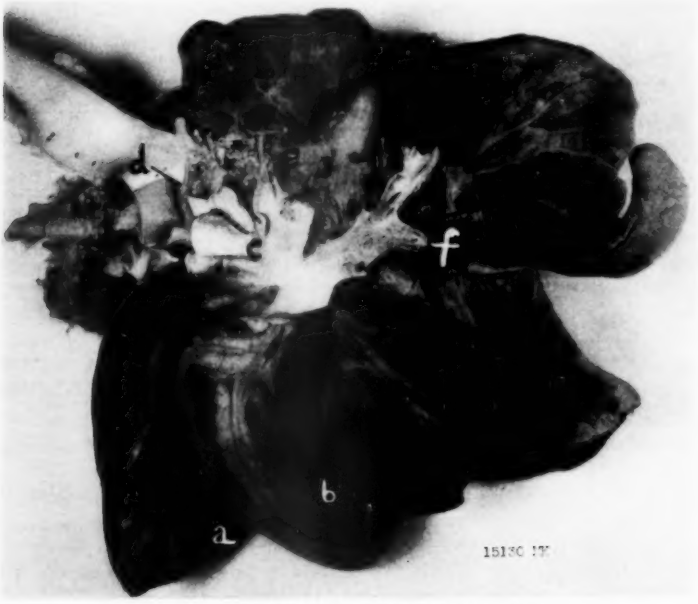

FIG. 1. PHOTOGRAPH OF GROSS MATERIAL

The huge right ventricle (*a*) is partially covered by the left ventricle (*b*). A probe is in the ductus arteriosus, extending from the pulmonary artery (*c*) to the aorta (*d*). The most constricted portion of the right pulmonary artery is seen at (*e*); and of the lower branch of the left pulmonary artery at (*f*). (Photographs by Milton Kougl.)

mal. Above this the pulmonary artery looked practically normal until the rough, rather wide, beginning of the ductus arteriosus was reached. The ductus arteriosus was patent, but as it approached the aorta it narrowed to a minute channel only 1 mm. in diameter. Beyond the orifice of the ductus arteriosus, the pulmonary artery divided in the usual manner. The lining of the right pulmonary artery, however, was roughened by trabeculae and markedly constricted. Indeed,

at the hilus of the lung it became so narrow that a probe could not enter its lumen. Beyond this stricture the three branches of the pulmonary artery continued into the lung as cord-like strands. The left pulmonary artery was also roughened by trabeculae which seemed to cause a thinning of the vessel wall rather than an increase in its thickness. At the hilus of the lung, the branch to the upper lobe seemed normally patent, but the lower branch was narrowed and occluded in a manner similar to that of the entire right artery.

The pulmonary veins were normal and emptied in normal fashion into a small left auricle. The mitral valve was entirely normal. The left ventricle was hypertrophied, but not proportionately to the right. Its wall measured 9 mm. in width. Its lining was smooth throughout. The aortic valve was normal and the aorta beyond, at first inspection, seemed unchanged except for an area of thickening and roughening at the point of entrance of the ductus arteriosus. On holding the aorta up to the light, one could see in its wall numerous thin zones, which resembled the pale trabeculae seen in the wall of the pulmonary arteries. The intimal lining was, however, smooth and showed no discoloration.

Lungs: In the left lung, the pressure of the enlarged heart had produced a large area of atelectasis. Before the viscera were removed prominently dilated vessels were noted at both lung bases, but these could not be traced because they collapsed after the lungs were taken from the body. The bronchial arteries were not traced before the dissection of the lungs; afterwards no widening of their lumina was noted.

Spleen: Weight 30 grams. It was quite hard and its surface was mottled by dark red patches.

Liver: Weight 500 grams. Its surface showed mottling, pale patches contrasting with darker ones. The dark red centers of adjacent lobules seemed to fuse together.

None of the other viscera showed any gross departure from the normal.

Culture of the heart blood was sterile. No organisms were seen in a direct blood smear.

On *microscopic examination of the tissues*, although attention was focussed on the large arteries and lungs, the following features were found to be worthy of note. The smaller arteries and their branches were normal in all respects. Sections of the carotids, the mesenteric, splenic and renal vessels revealed no lesions on careful examination. The pulmonary vein was also entirely normal. No lesions were found in the kidneys. The bones were entirely normal. There was slight scarring in the myocardium of the hypertrophied right ventricle and a typical mural thrombus was attached to its thickened endocardium. Its epicardium showed patchy areas of round-cell infiltration. Glycogen stains showed a large amount of glycogen in the cardiac muscle, particularly near the endocardium. It was also found in great quantities in striated muscle, but none was stained in the kidney. In the liver no glycogen was found, but this was probably due to the fact that the liver tissue had been greatly reduced by the severe grade

of chronic passive congestion. There was also extreme chronic passive congestion of the spleen. The thymus was atrophic and contained quantities of calcified Hassall bodies. Only two small parathyroids were dissected. They showed no microscopic lesion.

The *pulmonary artery* on section showed a most extraordinary calcification. Even in portions where grossly no obvious change was seen, the microscopic sections revealed a profound change. Starting with a section taken from the pulmonary conus, where, at most, there was only gross wrinkling, the artery showed patches of parallel fuzzy blue linear streaks in hematoxylin and eosin stains. In some places these streaks coalesced to form a nodule of calcification with narrowing of the media (Plate I, Fig. 5); otherwise the media seemed somewhat widened and the intima was definitely thickened. No cellular reaction occurred about these calcified foci. Although the internal elastic lamella was everywhere intact, elastic tissue stains demonstrated no elastica in the calcified areas of the media. The widening of the media was due to diffuse connective-tissue infiltration. In a more distal section of the pulmonary artery, there was an accentuation of this process, the linear calcification almost completely replacing the elastica. Here the nodules occurred at closer intervals. A section taken from the narrow branch of the left pulmonary artery at the hilus showed a greatly reduced lumen with a narrow wall composed of thickened intima and almost obliterated media in which were embedded numerous solidly calcified areas. Here, too, there was no cellular infiltration. The almost completely obliterated, though still patent, right pulmonary artery was seen to consist of a slightly thickened intima surrounded by a complete ring of calcification. This calcified ring was all that remained of the media (Plate I, Fig. 4). There was no remnant of elastica in this vessel.

The aorta showed a similar change in numerous sections taken from the arch, and the thoracic and abdominal portions; but the lesion was everywhere diffuse with no patches of solid calcification (Plate I, Figs. 2 and 3). The elastica could never be stained in these linear calcified areas, which, for the most part, occurred in the middle zone of media and extended toward the intima without ever reaching the internal elastic lamella. Where patches of elastica could be stained, the fibers seemed less wavy, almost straight and shorter than usual. Nowhere in the large arteries did the elastica stain as intensely as in the internal elastic lamella of the small vessels found in and about the adventitia. There was no indication of any fresh necrosis, nor was there any cellular inflammatory reaction. The internal elastic lamella could always be identified, but it stained lightly and showed a fraying of the fibers which occupied the intima. No calcification was seen along it, such as was described in Autopsies No. 8583 and No. 11723.

Fat stains of sections of the pulmonary arteries and aorta showed no fat accumulation in the thin portions of media. Nor could any fat be stained in the thickened intima. Levaditi stains revealed no spirochaetes in the arterial lesions, and Gallein stains demonstrated that the blue medial streaks were identical with

calcium. The nature of this material was further identified as calcium by its failure to stain after decalcifying in weak acid solution.

The extreme narrowing of the large pulmonary arteries caused remarkably little change in the lungs. The parenchyma showed only a few patches of haemorrhage with large pigmented cells in adjacent alveoli; and a few small foci of lobular pneumonia. In the right lung, particularly, and also in patches in the left, the adventitia of the small arterioles showed fibrous thickening. This made the vessels near the bronchi appear disproportionately large and conspicuous. In a few vessels the intima was thickened; in at least two small arterioles the lumen was completely obliterated. The media of the arterioles, however, showed no alteration. Elastic tissue stains showed normal elastica.

DISCUSSION

In the study of the autopsy material of the foregoing case particular attention was focussed upon the pronounced constriction or partial atresia of the branches of the pulmonary artery, and the unusual calcification of the media of these vessels. Similar, though less extensive, calcification was found in the main pulmonary trunk and also in the aorta. All of the small vessels except the pulmonary arterioles were normal. The heart showed right-sided enlargement and a patent foramen ovale and the cardiac muscle showed an increased glycogen content.

The demonstration of an increased amount of glycogen in the heart muscle raises the question whether the cardiac hypertrophy was due to von Gierke's disease. The glycogen content of the myocardium was not sufficient to justify that diagnosis. Furthermore, in von Gierke's disease there is generalized cardiac enlargement and not, as in this case, a predominant right-sided enlargement; nor is von Gierke's disease associated with arterial abnormalities. The increased glycogen content of the heart muscle was probably secondary to the great liver damage which had resulted from the severe, long-standing, chronic passive congestion.

The heart itself showed no primary malformation. All four chambers and all four valves were normally formed. The ductus arteriosus was not entirely obliterated but was of small size and probably not functioning. The foramen ovale was still patent. The elevated pulmonic blood pressure had evidently raised the pressure in the right auricle sufficiently to cause a right to left shunt through the foramen

ovale. The patency of the latter was, therefore, probably the result rather than the cause of the constriction of the pulmonary arteries.

This pronounced constriction of the branches of the pulmonary artery was the apparent cause of the tremendous enlargement of the right side of the heart. The deformity of the chest indicated that the cardiac enlargement had been of long duration. Since the stenosis of the pulmonary artery almost certainly antedated the cardiac enlargement, the former must have occurred in early infancy, if not in intrauterine life. There was no obvious cause for this narrowing of the pulmonary artery, neither recent infection nor old inflammation. It had the appearance of a congenital malformation of the artery.

The relation of the constriction of the pulmonary artery to the arterial calcification is not clear. Was the calcification the cause of the constriction of the pulmonary artery, or was it secondary to the constriction? The possibility exists that it was an incidental finding produced by an unknown etiological factor even though the calcification was most marked where the stenosis was most extreme. Although the recognized causes of arterial calcification appear to have been lacking in this case, the following are worthy of discussion.

Syphilis must be considered because it so frequently causes medial lesions in the aorta. Calcification of these lesions, however, is quite unusual. In this case, the blood Wassermann reaction of the patient and both his parents was negative. Levaditi stains revealed no spirochaetes in any of the affected arteries. No lesions suggestive of congenital syphilis were found. Therefore, we believe that syphilis is clearly excluded.

Hyperparathyroidism, as was mentioned in discussing Surbek's case, must also be considered. In the present case, the high nonprotein nitrogen in the blood was thought to be secondary to the cardiac failure. There was no clinical evidence of rickets or tetany and, therefore, no blood calcium or phosphorus determinations were made. At autopsy the kidneys and the bones appeared to be normal, and the parathyroids showed no lesion. The findings differed still further from those observed in cases of hyperparathyroidism in that the abnormal calcification was limited to the aorta and the pulmonary artery.

Viosterol, when given in massive doses, has been proved experi-

mentally to cause widespread calcification of the arteries. This patient did not receive abnormally large doses of viosterol and, furthermore, the calcification was not generalized.

Hypertension of the lesser circulation, so-called "Ayerza's disease," must also be considered. The essential pathological feature of this disease is obliterative pulmonary arteriolosclerosis (16), which primarily affects the intima of small arterioles in the lesser circulation. In our case, although there was slight thickening of the intima of a few of the pulmonary arterioles, the most extensive changes occurred in the media of the large arteries. Thus the pathological findings were totally unlike those observed in Ayerza's disease.

Maternal toxemia was considered by Jaffe as a possible cause of early calcification. In his case the mother suffered from hydramnios whereas in our case the mother suffered from pernicious vomiting of pregnancy. No other cases of pernicious vomiting have been reported in association with abnormal calcification in the fetus and, therefore, there is no reason to assume that there is any relation between the two conditions.

There remains the possibility that some congenital malformation was responsible for the abnormal arterial calcification. The lesion consisted of a defect in the medial elastica of the aorta and the pulmonary artery. The elastica failed to stain properly and appeared to be the primary site of the abnormality. At first the lesion consisted of patchy linear calcification of the individual fibers; these failed to take any elastic tissue stain. The uncalcified elastica was also altered. It had lost its spiral continuity and consisted of rather short straight rods which stained but faintly. The internal elastic lamella of the involved vessels, though not calcified, also took the stain faintly as though some constituent part was lacking. The coalescence of the areas of calcification caused an extreme medial defect. In these areas all of the elastic tissue had been lost. There was, however, no evidence of any degenerative process and no cellular reaction. The increase in the connective tissue appeared to be a compensatory phenomenon; this filled the defect where the vessel wall had been injured.

Thus, although it appears that the arterial calcification was in all probability due to some congenital defect in the media, the relation

of this defect to the constriction of the pulmonary artery remains obscure. There is at present no available method to determine whether the defect in the media was the cause or the result of the abnormal constriction of the pulmonary artery. That calcification is not inevitable, or indeed usual, in cases of arterial stenosis, is indicated by the fact that sections taken from several cases of extreme coarctation of the aorta revealed no calcification in the region of the stenosis. Winternitz (17), however, has reported that calcification may sometimes be found in the tissue of the obliterated ductus arteriosus.

Regardless of whether it was the primary defect, the constriction of the pulmonary arteries led to enlargement of the right side of the heart in much the same fashion as coarctation of the aorta leads to enlargement of the left side. In this connection it must be borne in mind that there is no absolute proof that coarctation of the aorta *of the adult type* arises during intrauterine life. The lesion in our case, if it did not arise prenatally, at least dated back to early infancy and, in the present state of our knowledge, it seems proper to classify it among the congenital malformations of the cardiovascular system which lead to enlargement of the heart.

SUMMARY

A case of stricture of the right pulmonary artery and of the lower branch of the left pulmonary artery in an infant 17 months of age is reported. Only 5 cases of congenital stricture or atresia of a branch of the pulmonary artery were found in the literature; in none of the cases was the lesion bilateral.

On microscopic section the entire pulmonary artery as well as the aorta showed extensive calcification of the media. The process was most extensive in the region where the constriction of the pulmonary artery was most pronounced. No etiological factor was found which would explain the calcification. Five other cases of arterial calcification occurring in infancy were found in the literature and three cases were discovered in the autopsy records of this hospital. In none of these was there any stenosis of the arteries.

In the present case, the stricture of the pulmonary artery was so severe that it put a strain upon the right side of the heart similar to that put upon the left side of the heart by coarctation of the aorta.

I wish to express my sincere appreciation to Dr. Helen B. Taussig for her aid in furnishing the clinical data for this report.

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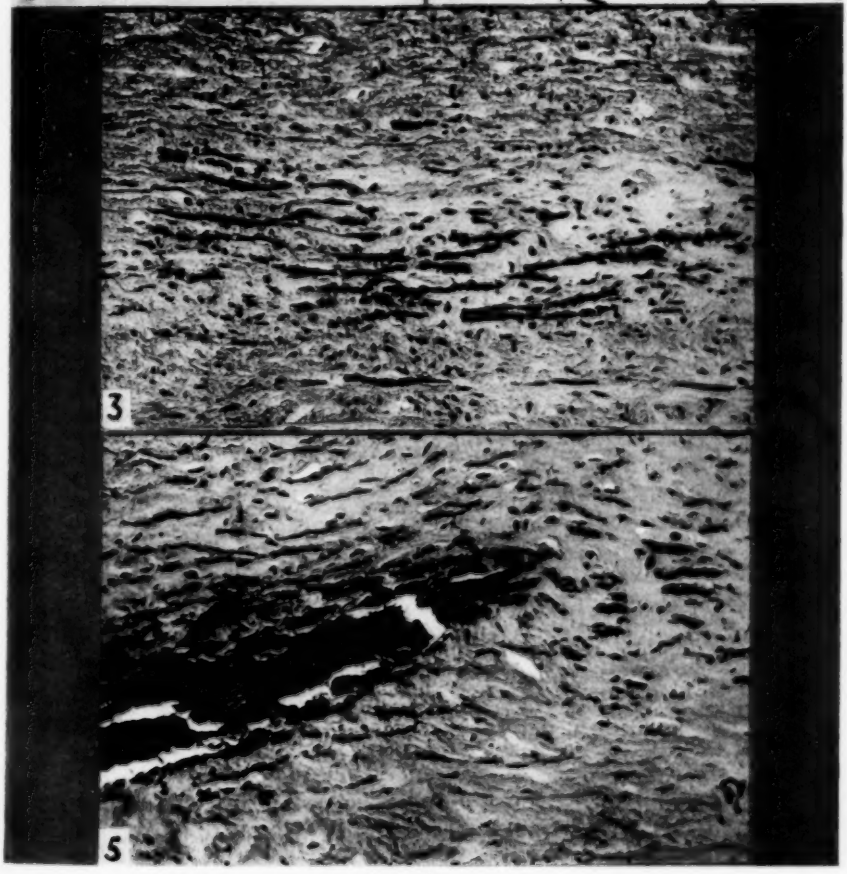
PLATE I

FIG. 2. Low-power magnification (×75) of aorta, showing the connective-tissue thickening of the intima (*a*); the media (*b*) with broken and calcified elastica and general thinning of this portion of its coat; delicate adventitia (*c*).

FIG. 3. High-power magnification (×200) of above, showing only the media with its calcified lines of elastica.

FIG. 4. Low-power magnification (×75) of right pulmonary artery at its most constricted portion, labeled (*e*) in figure 1. A postmortem clot fills the lumen; the intima (*a*) is barely thickened, but is torn loose from the media (*b*) which consists of a solid ring of calcium.

FIG. 5. High power magnification (×200) of the media of the pulmonary artery just above the conus, showing the edge of a large calcified plaque which has obviously been produced by the coalescence of many individual calcified elastic fibres (*a*).



UNIV. OF MICH.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

A Textbook of Hematology. By WILLIAM MAGNER, M.D., D.P.H. 395 pp. Illus. $4.50. (*P. Blakiston's Son & Co., Inc., Philadelphia, 1938.*)

Dr. Magner has evidently prepared this short textbook for the purpose of giving the medical student and practitioner a brief survey of the field of hematology.

Seven of the seventeen chapters deal with the characteristics of normal blood cells and the methods employed in hematology. The monophyletic and polyphyletic theories as to the origin of the blood cells are discussed with the aid of Wiseman's diagrams. Sternal puncture is advocated as a means of studying the bone-marrow during life, whereas actual biopsy of the marrow, which many feel may give more valuable information, is not stressed. The author fails to mention the methods for calculating the mean corpuscular volume, the mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration which are now so widely employed that they deserve a place in every textbook of hematology. The chapter devoted to hemoglobin and its derivatives is to be commended as this is a subject which has received little attention in the past.

The classification of the anemias as post-hemorrhagic, hemolytic and dyshemopoietic is rather unsatisfactory, in that a great variety of the anemias including the pernicious type and related macrocytic anemias, aplastic anemia, hypochromic anemias apart from those due to blood loss, and the anemias of infancy are thrown together into the heterogenous dyshemopoietic group. Polycythemia and the disorders of the leucocytes—leukemias, agranulocytosis, and infectious mononucleosis—are discussed very briefly with the aid of illustrative case reports.

By limiting the bibliography to some 200 odd references, taken entirely from the British and American literature, the author fails to present as broad a view of the field of hematology as would be desirable in a book of this nature.

The book is well printed on good paper in large legible type so that it reads very easily. The photomicrographs are quite good, but the color plate illustrating the various types of blood cells is rather poor.

W. H. B.

Physicians and Medical Care. By ESTHER LUCILE BROWN. 202 pp. 75 cents. (*New York, Russell Sage Foundation, 1937.*)

This monograph presents in a concise form information concerning the profession of medicine and provisions for medical care in the United States. Almost one-half of the book is devoted to a survey of medical education. The remainder

deals with the national associations concerned with medicine, the number, distribution and income of physicians, new forms of medical service, "health insurance" and "state medicine." The author concludes that the problem of the twentieth century is "that of devising ways and means whereby adequate care can be provided for the entire population and physicians compensated fairly for the service rendered." She admits that the task is not easy and does not undertake to tell us how it is to be done, but leaves the problem squarely on our door step. The book contains much useful information and appears to be written without bias.

A. M. C.

Textbook of Medicine. By American Authors. Edited by Russell L. Cecil. 4th ed., revised and reset. 1614 pp. Illus. $9.00. (*W. B. Saunders Company, Philadelphia, 1937.*)

From the time of the initial publication of this textbook a decade ago, it has received favorable recognition. The present fourth edition of it attests to a continuing appreciation of its worth. In order to have "the advantage of introducing new blood into the textbook and of interesting the younger group of University teachers in maintaining the present high standard of its contents," many new contributors replace some of the original roster. A number of new topics have been introduced, such as Lymphogranuloma Inguinale, Several New Virus Infections, Pneumonia in Childhood, Staphylococcus Infections, Meningococcal Sepsis, Spontaneous Hypoglycemia, The Nephroses, Classification of Diseases of the Heart and Diseases of the Peripheral Vessels, and not only have all subjects been brought up-to-date but many of them have been presented by new authors.

The book is valuable to students, to practitioners and to teachers. Its subject-matter is dealt with concisely and accurately and is made doubly serviceable by a good index.

C. R. A.

A Bibliography of the Works of Ambroise Paré. By Janet Doe. 266 pp. Illus. $5.00. *University of Chicago Press, Chicago, 1937.*

To appreciate properly the work of such an influential writer as Ambroise Paré it is necessary to have considerable knowledge of the bibliography of his works. Thus, for example, it is highly important to know that the 17th century English edition of his *Workes* was taken not from the original French version, but from a rather faulty Latin translation. In many instances the chronology of various editions of his writings gives a clearer view of the development of the thought of the author.

Since the collected edition by Malgaigne in 1840, with its valuable bibliography, there has been no adequate study of this phase until the appearance of Miss Doe's volume. She has had the advantages of many resources not available to Malgaigne and has described the books in much more detail, giving the full titles, collation, contents, errata, illustrations, and other pertinent information. There are repro-

ductions of the title-pages of all the important editions. A valuable feature is a census of copies in a great many libraries throughout the world.

In addition to the technical descriptions, she tells a great deal of the matter of the books and of their influence, and is therefore able to give us really good reading and in this sense much more than the usual dry bibliography. The printing and illustrations are excellent, and the book will be of inestimable value not only to the collector but also to those interested in the history of medicine and in the general history of this period.

S. V. L.

Scientific Contributions in Honor of Joseph Hersey Pratt on his sixty-fifth birthday. By his Friends. 983 pp. Illus. Lancaster Press, Lancaster, Pa., 1937.

In the introduction to this Festschrift "by his friends," it is said of Dr. Pratt that "he has served as an example of what a physician in practice can do in advancing the science of medicine"; this is eminently true. It is equally true that the eighty contributions from this country and abroad, many of them by men of eminence, attest the genius for friendship that is Dr. Pratt's. The modest hero of the occasion is a man of unusually broad interests; the delightful *Appreciation by Dr. Richard Cabot* will give the reader a clue to why the scope of subjects—scientific, historical and humane—are fitting to the occasion.

Many of the chapters appeared recently in the Annals of Internal Medicine; some emanate from Germany—by Von Mueller, Korth, Strieck, Dennig, Brugsch, Grafe and Tropp. Krehl and Morawitz died before making their expected contributions. England is represented by Sir Humphry Rolleston and Sir Arthur Hurst. As is proper, Boston is strongly represented; but, in addition, many other chapters make the symposium truly representative of American medicine. Some are of the very first importance. Most of them are sufficiently vital to escape the appearance of being exercises in writing rather than contributions to medical investigation. On the whole, the volume can be considered a very valuable spectrum of contemporary medicine and an earned tribute to a fine figure in American life.

J. T. K.

BOOKS RECEIVED FOR REVIEW

A Biological Approach to the Problem of Abnormal Behavior. By MILTON HARRINGTON. 459 pp. $4.00. Science Press Printing Co., Lancaster, Pa., 1938.

On a New Gland in Man and Several Mammals (Glandulae Parathyreoideae). By IVAR SANDSTRÖM. [Upsala Läkareförenings Förhandlingar, 1879–80, *15*, 441–471] Translated by Carl M. Seipel. Edited by Charlotte H. Peters and J. F. Fulton. With biographical notes by Professor J. August Hammar. 44 pp. Illus. $1.00. The Johns Hopkins Press, Baltimore, 1938.

On Thought in Medicine (Das Denken in der Medizin). By HERMANN VON HELMHOLTZ. An Address delivered August 2, 1877, on the Anniversary of the Foundation of the Institute for the Education of Army Surgeons. Introduction by Arno B. Luckhardt. 27 pp. 75 cents. The Johns Hopkins Press, Baltimore, 1938.

Pneumonia and Serum Therapy. By Frederick T. Lord and Roderick Heffron. Revised edition of Lobar Pneumonia and Serum Therapy. 148 pp. Illus. $1.00. The Commonwealth Fund, New York, 1938.

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Anatomy and Physiology Laboratory Guide. By EDMOND J. FARRIS. 2nd ed. 112 pp. Illus. J. B. Lippincott Company, Philadelphia, 1937.

Bile: Its Toxicity and Relation to Disease. By O. H. HORRALL. 434 pp. $4.00. University of Chicago Press, Chicago, 1938.

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Symptoms of Visceral Disease. By FRANCIS MARION POTTENGER. 5th ed. 442 pp. Illus. $5.00. C. V. Mosby Company, St. Louis, 1938.

Leukemia and Allied Disorders. By CLAUDE E. FORKNER. 333 pp. Illus. $5.00. The Macmillan Company, New York, 1938.

Syphilis, Gonorrhea and the Public Health. By NELS A. NELSON & GLADYS L. CRAIN. 359 pp. $3.00. The Macmillan Company, New York, 1938.

Fifty Years a Country Doctor. By WILLIAM N. MACARTNEY. 584 pp. $3.50. E. P. Dutton & Co., New York, 1938.

THE EFFECT OF PREVENTING THE DEVELOPMENT OF HYPERSENSITIVITY IN EXPERIMENTAL TUBERCULOSIS

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In recent years much information has accumulated on the relationship of hypersensitivity to immunity in various infections. Many investigators have come to question the beneficial rôle of hypersensitivity, and some have taken the view that the development of the hypersensitive state is distinctly deleterious to the organism.

In tuberculous infection Rothschild, Friedenwald and Bernstein (1) were able to separate hypersensitivity from immunity. They infected guinea pigs with attenuated tubercle bacilli in order to make them hypersensitive and immune. Desensitization was then accomplished by administering gradually increasing doses of Old Tuberculin[1] until the animals tolerated 2 cc. of undiluted O. T. a day. After reinfection with virulent bacilli the animals were maintained in a continuous state of desensitization by daily injections of 2 cc. O. T. In comparison with hypersensitive controls, the desensitized animals showed an unimpaired immunity as measured by "the course and severity of local lesions, by the spread and severity of their visceral lesions, and by the mortality." These results have been confirmed by others (2–7).

Since by this means hypersensitivity and immunity in tuberculosis have been separated, it seemed worth while to study the extent and character of the lesions in infected animals that were prevented from ever developing hypersensitivity. This could be accomplished by administering large amounts of O. T. daily from the day of primary infection to the day of death. It was with this in mind that the

[1] Hereafter written O. T.

present study was undertaken. Since this present work was begun, Willis and Woodruff (8) have reported experiments which they feel indicate that "animals which have been prevented from developing allergy by injection of tuberculin, are unusually susceptible to tuberculosis and develop what is probably the most marked degree of tuberculous pneumonia yet produced in experimental animals."

MATERIALS AND METHODS

Adult male guinea pigs weighing between 400 and 600 grams were used. These were kept in an uncrowded environment and received an unlimited supply of food, consisting of "Rab-ets" and carrots.

The infecting strain of tubercle bacilli was isolated from the sputum of a patient with active pulmonary tuberculosis several years ago. In our experience it has proved to be extremely virulent for the guinea pig, and over a period of two years it has not declined in virulence. It has been kept growing on Proskauer and Beck's synthetic medium adjusted to pH 7.4. In making up the infecting suspension a small piece of pellicle from a 3- to 4-week culture was well ground with mortar and pestle; 0.9% saline was then introduced very slowly, drop by drop, the whole process taking at least 20 to 30 minutes, until about 5 cc. had been added. This suspension was lightly centrifugalized, and a loopful of the supernatant smeared over an area 1 cm. square on a slide containing a thin film of egg albumen. This was stained with carbol fuchsin and examined with the oil-immersion lens. Most of the bacilli were single, although a few small clumps could be seen. The number of organisms in 20 oil-immersion fields was counted; dilutions were made until the desired number of bacilli was obtained. 0.1 cc. of the total suspension was injected subcutaneously into the groin using a 21-gauge needle.

The O. T. used throughout the experiment was of two kinds. The one used in Series I and in large part in Series II was made from Proskauer and Beck's synthetic medium on which our "sputum strain" of human virulent organisms had grown for 8 weeks. In Series II, tuberculin was used which was made from human virulent strains "C," "D. T." and "P. N." (obtained from the U. S. Bureau of Animal Industry) grown on Dorset's liquid medium. In preparing the tuberculin, the media and bacilli were filtered by suction through

paper. The filtrate was then passed through a Berkefeld N candle and evaporated to one-tenth the original volume on the steam bath. As a precipitate formed, the concentrated tuberculin was refiltered through paper, bottled, reheated to 100°C. for half an hour, and finally stored in the ice-chest. When tested on tuberculous guinea pigs, both tuberculins in 1 mgm. doses evoked a positive skin reaction.

The tuberculin was injected subcutaneously. For this purpose, the hair over the right side of the body was clipped and the injections were made on this side only for the entire experiment. The opposite side was reserved for skin tests. 20-gauge needles 1 inch long, with short bevels, were used. After some practice, it was found that if, after injecting the material, the needle was withdrawn very, very, slowly, no liquid leaked out.

Skin tests for hypersensitivity were made at intervals, as described below. The tests were read at 24, 48 and 72 hours.

Blood was removed from the animals just before death by cardiac puncture. The sera obtained were kept frozen at minus 20°C. Before use, the sera were inactivated at 56°C. for 20 minutes.

For the complement-fixation reaction, the antigen was a suspension of heat-killed virulent human bacilli, the strain used for infection. This was titrated to determine its affinity for complement, and diluted appropriately. The complement was guinea pig serum diluted 1:10. The hemolytic system consisted of sheep cells and anti-sheep cell rabbit serum. Normal serum controls were used in each set of tests, and tubes containing no antigen were also set up to test the anti-complementary power of the serum. The antigen, complement and serum mixtures were kept in the ice-box for 8 hours. Then, after remaining at 37°C. for 30 minutes the hemolytic system was added and the results were read.

In testing agglutinins, the method described by Mudd (9) was used. Controls of normal serum were set up with each series. The antigen was a suspension of unheated virulent tubercle bacilli of the infecting strain.

The animals were autopsied as soon as possible after death. Tissues from Series I were fixed in Zenker-formol and microscopic sections were made of spleens, livers, lungs and lymph nodes and stained with hematoxylin and eosin. Sections of lungs and spleens were stained

with carbol fuchsin. The organs of Series II were fixed in Kaiserling I and II, so that photographs could be made. The tracheo-bronchial lymph nodes were dissected under binocular magnification. Sections were made of lung, lymph nodes, spleen and liver from each animal. *Each section of lung comprised a complete longitudinal section of the organ from apex to base.* All were stained with hematoxylin and eosin and with carbol fuchsin. When stains for bacteria were made, the technique of Brown and Brenn (10) was used.

EXPERIMENTAL RESULTS

Series I consisted of 16 animals. Eight were given daily subcutaneous injections of 1 cc. undiluted O. T. for 10 days. After this 2 cc. O. T. were given each day until the death of the animal. The remaining 8 animals received nothing and served as controls. On the 4th day of the tuberculin treatment all 16 animals were infected in the left groin with a suspension of the "sputum strain" of tubercle bacilli containing 5–6 organisms per oil-immersion field. All were tested intracutaneously with 50 mgm. O. T. at 20 and 37 days after infection and the survivors were tested intracutaneously at 40 days with a suspension of the "sputum strain" tubercle bacilli.

Table I shows how long each animal lived, how much O. T. was given, and the percentage weight gain or loss, together with the gross extent of the lesions in lungs, spleen, and livers, graded from 1 to 4. The number of acid-fast organisms in lungs and spleens and the degree of caseation have also been tabulated. In the Table, *1* indicates lesions composed of epithelioid cells and tubercles with no caseation; *2* indicates the above, together with areas of beginning caseation; *3* and *4* indicate more extensive lesions with marked caseation. In grading the number of organisms, *1* indicates only a few scattered acid-fast bacilli in the entire section. In *2* the number increases and a few organisms are seen in almost every high-power field containing no lesions. *3* indicates a still larger number of bacilli, averaging 15–20 in each high-power field, while *4* comprises those sections in which there are enormous numbers of bacilli which can readily be seen under low magnification.

It will be seen that 5 treated animals and 4 controls lived into the 4th week, while 3 treated and 4 controls lived 42 days. At autopsy

no appreciable differences were found macroscopically in the extent of the lesions in the two groups. Microscopically no distinct differences could be noted in the extent and character of the lesions in the O. T.-treated and control animals dying in the 4th week. Those coming to autopsy in the 6th week showed a marked difference between the treated animals and the controls. In spite of the fact that there was no appreciable difference macroscopically between the lesions in the two groups at this time, microscopically the cha racter

TABLE I*

ANIMAL NUMBER	LENGTH OF LIFE	TOTAL OLD TUBERCULIN	CHANGE IN WEIGHT	LUNGS			SPLEEN			LIVER	
				Gross	Caseation	Bacilli	Gross	Caseation	Bacilli	Gross	Caseation
	days	*cc.*	*per cent*								
57	22	40	−4	1	1	1	1	2	1	0	1
56	22	40	−9	0	0	0	1	2	1	0	1
60	22	0	−15	0	1	1	2	2	1	0	1
69	22	0	−6	1	1	1	2	2	1	0	1
62	24	44	−1	0	0	0	2	2	1	0	0
66	24	0	−4	1	1	1	2	2	1	1	1
72	25	46	−3	0	1	1	1	2	1	0	1
68	25	0	+4	0	1	1	1	2	1	0	1
61	28	52	−16	1	1	1	2	2	1	1	1
65	42	80	−18	3	3	3	2	2	1	1	2
63	42	80	−18	4	3	4	3	2	1	2	2
67	42	80	−26	3	3	4	3	3	2	2	2
70	42	0	+3	2	3	1	2	2	1	1	1
58	42	0	−2	3	3	2	3	2	1	2	3
71	42	0	+4	2	2	1	3	2	1	3	2
55	42	0	+12	4	3	1	3	3	1	2	2

* See text for explanation of numbers.

of the lesions in the treated animals was very different from that of the controls. In the former there were in the lungs many alveoli plugged with polymorphonuclear and mononuclear leucocytes together with cellular débris. The alveolar walls were usually intact but some showed interstitial infiltration with mononuclear and a few polymorphonuclear cells. When these sections were stained with carbol fuchsin, an amazing picture presented itself, for in such areas the exudate in the alveoli was teeming with acid-fast organisms, so many, in fact, that they could be seen with the low-power objective.

Such lesions were not present in the 4 control animals. The livers and spleens of both groups showed about the same degree of caseation, and relatively few bacilli were found in these organs.

As noted above, the animals in this series were tested at varying intervals, intracutaneously, with O. T. and with bacilli. All eight of the animals receiving O. T. gave negative reactions for all tests while the control animals showed at each test positive reactions with necrosis both to tuberculin and to the bacilli.

In the experiments of Willis and Woodruff (8) the lesions of the character described above which contained so many bacilli occurred in the tuberculin-treated animals, and their severity was attributed to the absence of hypersensitivity. However, since the controls in both Willis and Woodruff's experiments (8) and those in this first series of ours received no treatment at all, it was thought desirable to control the factor of daily trauma by giving the animals, which did not receive tuberculin, control injections of other fluids so that they would have to undergo the same handling and trauma. A second series was carried out for this purpose.

Series II consisted of 61 animals that lived over 5 weeks (36 to 80 days). All were infected in the left groin with a suspension of "sputum strain" bacilli containing 20 organisms per oil-immersion field. Treatment was begun on the day of infection. Eighteen animals received daily subcutaneous injections of O. T., one-half receiving 1 cc. each day, the rest receiving 1 cc. daily during the first week, 1.5 cc. daily during the second week and 2 cc. each day from then on. Since O. T. contains glycerin, 21 received injections of 10% glycerin in saline, 9 receiving 1 cc. each day while 12 were given 1 cc. each day for the first week, 1.5 cc. every day of the second week, and 2 cc. each day thereafter. The remaining 22 animals received daily injections of 0.9% sodium chloride solution, 12 receiving 1 cc. and 10 1 cc. the first week, 1.5 cc. the second week and 2 cc. from then on.

All animals were tested intracutaneously with O. T. 21, 35, and 46 days after infection, the controls receiving 10 mgm. while the O. T.-treated animals received 100 mgm. All were given intracutaneous tests with a suspension of heat-killed tubercle bacilli of the "sputum strain" at 41 and 63 days after infection and 6, 2 from each group (Nos. 143, 145, 176, 159, 183, 166) were tested with live tubercle

bacilli of the same strain at 56 days after infection. The O. T.-treated group gave no positive reactions, while the two control groups gave positive necrotic reactions to both the tuberculin and the bacilli (Figs. 1, 2, 3).

Table II contains the pertinent data dealing with this series of animals. In it are listed the number of days each lived, the total material received, the condition of the animal at the time of death, and the percentage of weight gained or lost during the experiment. Shown, too, are the lesions and number of bacilli graded as in the preceding series and also the antibody titers of those animals from which blood was obtained.

It will be seen that animals came to autopsy from the 36th to the 80th day after infection. The majority of the animals lost weight during the experiment, as might have been expected; however, there are no very significant differences in average weight loss per animal from each group. The O. T.-treated group lost an average of 12% of the initial weight per animal while in the glycerin and saline controls the average loss was 16% and 13%, respectively. The condition of the animals varied a great deal. Some even gained weight during the experiment while others became moribund, losing as much as 150–200 gms. in a week.

It will be seen from Table II that the severity of the lesions varied widely. On the whole, however, there was distinct difference between the two groups of animals treated with O. T. and with glycerin as compared with the saline treated controls. In the latter group the lesions were distinctly less extensive than in the two former. In regard to the relative extent of the lesions in the lungs, livers, and spleens in each animal of the various groups there was a great deal of variation. Taking all the animals, however, they could be divided into those showing equal involvement of lungs, livers and spleens, and those in which the lungs were either more or less involved than their respective livers or spleens. In the guinea pig, as is well known, the spleen and liver are ordinarily much more involved in tuberculosis than are the lungs. It is remarkable, therefore, that in about one-third of all of the animals in this series the lungs showed decidedly more tuberculosis than the liver or spleen. The heavier involvement of the lungs was not limited to the non-hypersensitive group. It

TABLE II‡

ANIMAL NUMBER	LENGTH OF LIFE	MATERIAL PER DAY*	TOTAL CC.	CONDITION AT DEATH†	CHANGE IN WEIGHT	LUNGS			SPLEEN			LIVER			ANTIBODY		HYPERSENSITIVITY
						Gross	Caseation	Bacilli	Gross	Caseation	Bacilli	Gross	Caseation	Bacilli	Complement fixation	Agglutination titer	
	days	*cc.*			*per cent*												
148	36	2 O.T.	61.5	D.	−20	0	1	2	1	2	1	0	1	1			0
196	36	2 G.	61.5	M.	−18	2	2	2	2	3	2	1	2	1	1–3	1:40	+
161	36	1 S.	36	D.	−38	1	2§	2	1	2	1	0	2	1			+
156	36	1 S.	36	M.	−20	0	2	2	1	2	1	0	1	1	1–6	0	+
144	37	2 O.T.	63.5	M.	−28	1	2	4	0	2	3	0	2	1	1–2	1:20	0
186	37	2 G.	63.5	D.	−24	1	2§	4	2	2	2	0	2	1			+
165	37	2 S.	63.5	M.	−35	1	2	2	2	2	2	0	1	1	0	1:20	+
135	37	1 O.T.	37	M.	−32	1	2	4	3	2	2	1	2	1	0	1:20	0
177	37	1 G.	37	K.	−16	1	2	2	2	3	1	0	2	1	0	0	+
158	37	1 S.	37	K.	−10	1	2	3	1	3	1	0	2	1	0	1:20	+
142	39	2 O.T.	67.5	M.	−33	2	3	1	1	2	1	2	2	1	1–8	1:40	0
185	39	2 G.	67.5	M.	−22	1	3	3	2	2	1	2	2	1	0	0	+
171	39	2 S.	67.5	K.	−15	1	2	4	2	3	2	0	2	1	0	0	+
167	42	2 S.	73.5	M.	−37	1	3	1	2	2	1	2	2	1	0	0	+
136	43	1 O.T.	43	M.	−24	2	3	4	2	3	2	1	2	1	1–8	1:80	0
187	43	1 G.	43	K.	−12	3	3§	2	2	2	1	0	2	1	0	1:40	+
151	43	1 S.	43	M.	−33	2	3	2	1	2	1	1	2	1			+
147	49	2 O.T.	87.5	M.	−28	4	3	4	0	2	3	0	1	2	1–6	1:40	0
178	49	2 G.	87.5	K.	−29	4	4	4	2	2	3	2	3	2	1–14	1:40	+
194	49	2 G.	87.5	K.	−21	3	3	3	2	2	2	0	2	1	0	1:20	+
173	49	2 S.	87.5	K.	−5	2	3§	1	1	2	1	0	1	1	1–6	1:160	+
198	51	2 G.	91.5	D.	−4	3	2	2	3	2	1	2	3	1			+
169	52	2 S.	93.5	D.	−27	3	4	4	2	1	1	2	2	2			+
189	54	1 G	54	M.	−20	4	4	4	2	2	1	3	3	2	0	1:80	+
140	55	2 O.T.	99.5	D.	−20	3	4	4	2	2	3	0	1	1			0
162	55	1 S.	55	D.	−30	3	3	3	2	3	1	0	2	1			+
143	58	1 O.T.	58	K.	+14	3	3	2	1	2	1	0	1	1	1–4	1:80	0
176	58	1 G.	58	K.	−20	2	3	2	2	3	1	1	2	1			+
159	58	1 S.	58	K.	−14	2	3	2	2	3	1	1	3	1	1–12	1:160	+
145	58	2 O.T.	105.5	K.	−2	2	3	2	0	1	1	0	0	0	1–3	1:160	0
183	58	2 G.	105.5	K.	−7	2	3	2	2	2	1	1	2	1	0	1:40	+
193	58	2 G.	105.5	D.	−36	3	2	2	2	3	1	2	3	1			+
166	58	2 S.	105.5	K.	0	2	3	1	2	2	1	1	3	1	0	1:40	+
192	60	2 G.	109.5	M.	−20	3	3	4	3	2	1	2	3	1			+
170	62	2 S.	113.5	D.	−14	3	4	4	2	3	1	3	3	1			+
133	65	1 O.T.	65	M.	−32	4	4	2	2	2	1	0	1	1	1–6	1:80	0
139	65	1 O.T.	65	K.	−5	3	3	2	3	3	1	2	3	1	1–8	1:40	0
179	65	1 G.	65	M.	−34	4	4	4	1	2	1	2	3	1	0	1:20	+

* O.T., Old Tuberculin; S., Saline; G., Glycerine.

† M, Moribund; K, Killed; D, Died.

‡ See text for explanation of numbers.

§ Secondary pneumonia.

4 Lesions showing enormous numbers of bacilli.

TABLE II—*Concluded*

ANIMAL NUMBER	LENGTH OF LIFE	MATERIAL PER DAY*	TOTAL CC.	CONDITION AT DEATH†	CHANGE IN WEIGHT	LUNGS			SPLEEN			LIVER			ANTIBODY		HYPERSENSITIVITY
						Gross	Caseation	Bacilli	Gross	Caseation	Bacilli	Gross	Caseation	Bacilli	Complement fixation	Agglutination titer	
	days	*cc.*			*per cent*												
188	65	1 G.	65	K.	−10	3	3	2	3	2	1	2	3	1			+
154	65	1 S.	65	K.	−6	2	3	2	4	3	1	2	3	1	1–14	1:80	+
164	65	1 S.	65	D.	−24	2	3	3	3	3	1	3	3	1			+
157	66	1 S.	66	M.	−8	3	3§	1	1	1	1	0	1	1	0	0	+
150	67	2 O.T.	123.5	D.	−12	2	3	4	3	2	1	1	2	1			0
190	67	2 G.	123.5	K.	−5	3	3	3	1	2	1	2	1	1	1–2	1:40	+
172	67	2 S.	123.5	K.	−2	2	3	3	2	2	1	1	3	1	0	0	+
146	68	2 O.T.	125.5	M.	−11	4	4	4	2	1	2	1	1	2	1–3	1:80	0
197	68	2 G.	125.5	K.	−2	4	4	3	3	3	1	2	3	1	0	1:40	+
174	68	2 S.	125.5	K.	+25	0	1	1	1	2	1	1	1	1	0	1:20	+
138	73	2 O.T.	135.5	K.	−17	0	2	1	1	2	1	1	1	1	1–3	1:20	0
191	73	2 G.	135.5	K.	+13	4	4	3	2	2	1	3	2	1	1–8	1:80	+
160	73	2 S.	135.5	K.	+10	2	2	1	2	2	1	2	2	1	0	1:20	+
141	73	1 O.T.	73	K.	−5	4	4	3	0	1	1	0	1	1	0	1:20	0
132	73	1 O.T.	73	K.	−8	4	4	2	0	1	1	0	1	1	1–4	1:40	0
175	73	1 S.	73	K.	−27	3	4	3	2	2	1	2	2	1			+
182	73	1 G.	73	D.	−22	1	4	4	3	2	1	4	3	2			+
152	73	1 S.	73	K.	+4	3	3	1	2	2	1	1	2	1	1–12	1:640	+
137	80	1 O.T.	80	K.	+35	2	2	1	1	1	1	0	1	1	1–6	1:160	0
134	80	1 O.T.	80	K.	+26	2	2	2	1	1	1	0	0	1	0	1:80	0
181	80	1 G.	80	K.	−5	3	3	1	2	2	1	2	2	1	1–112	1:160	+
184	80	1 G.	80	K.	−12	2	3	2	2	2	1	1	2	1	1–14	1:160	+
153	80	1 S.	80	K.	+4	2	2	1	2	2	1	2	1	1	1–64	1:320	+

occurred in the glycerin-treated and in the saline-treated hypersensitive groups as well. It may be further stated that the preponderance of lesions in the lungs bore no constant relation to the number of bacilli found in the lesions. In the non-hypersensitive group only half showed lesions in the lungs which were more extensive than in the livers or spleens. Half showed either equal or more involvement of the spleen and liver than of the lung.

In all animals of all the groups the spleen showed tuberculous lesions. This is in contrast to the findings of Willis and Woodruff (8) who found that in the desensitized animals the spleen showed "no definite tubercle formation."

When one examines the lungs of our animals to determine the de-

gree of caseation, there is a tendency for less caseous change to be present in the O. T.-treated animals than in the other two groups.

As far as the number of stainable acid-fast bacilli in the various organs was concerned, a wide variation existed also. Some of the animals in each group showed no more bacilli in their lesions than do similar untreated guinea pigs; others showed a definite but moderate increase in numbers of bacilli; and in 7 of the 18 non-hypersensitive O. T.-treated animals (39%), in 6 of the 21 hypersensitive glycerin treated animals (28%), and in 3 of the 22 hypersensitive saline controls (13%), lesions were found containing enormous numbers of bacilli identical with those described in Series I (see illustrations). In such animals the liver and spleen showed lesions and bacilli could be stained, but they were few in comparison to the number in the lungs.

As regards the development of antibody in the three groups of animals, it will be seen that of the 44 sera tested, 24 or 54% gave positive complement fixation in varying titer, and 37 or 84% gave positive agglutination in titer up to 1:640. What significance these antibodies may have will be discussed below.

DISCUSSION

In experiments of the type performed by Rothschild and his colleagues (1), in which hypersensitive and immune animals were desensitized and then reinfected, there was an obvious difference in the character of the resulting lesions when compared with hypersensitive controls. In the former group the lesions showed less necrosis. This difference has also been noted by others, including Birkhaug (6) and Higginbotham (7). In our experiments, as noted above, there was a distinct tendency for the lesions of the non-hypersensitive infected animals to show less caseation than those of the controls. We have also found this same difference in another experiment of a similar type (11). Since in tuberculosis much of the necrosis and caseation is dependent on the hypersensitive state, it is not surprising to find less necrosis and caseation in the non-hypersensitive animals, such as was noted in this experiment.

We have now to consider the factors which might conceivably play a rôle in the production of the peculiar pulmonary lesions containing

such enormous numbers of acid-fast organisms. In Series I this change occurred only in animals of the O. T.-treated group and was not seen in the non-injected controls. Since the treated animals were not hypersensitive while the controls were, it seemed not unlikely that, as Willis and Woodruff (8) concluded, this free and unrestrained growth of tubercle bacilli in the lung was the result of the lack of hypersensitivity. However, in both Willis and Woodruff's experiments (8) and in this first series of our own, the control animals had received no treatment and were spared the handling and trauma incident to the daily injections to which the test animals were subjected. The results in our second series of animals, in which appropriate controls of this nature were carried out, failed to bear out the assumption that the marked proliferation of the bacilli was due to a lack of hypersensitivity for, in the first place, the lesion occurred in only 39% of the O. T.-treated non-hypersensitive animals and was not found in any of the remaining 61% of the animals that lacked hypersensitivity; and, on the other hand, it did occur in 28% of the hypersensitive glycerin controls and in 13% of the hypersensitive saline controls. Lack of hypersensitivity cannot therefore explain the great proliferation of bacilli in the pulmonary tissues. All of the animals treated with O. T. were shown to be non-hypersensitive by intracutaneous tests with both tuberculin and heat-killed tubercle bacilli. If lack of hypersensitivity were the important factor in permitting the free growth of bacilli, it seems unlikely that only about one-third of these non-hypersensitive animals would have exhibited this peculiar change, regardless of the length of time they lived. Furthermore, if one postulates that hypersensitivity can prevent this excessive proliferation of bacilli, how can one explain the occurrence of this phenomenon in 6 of the 21 glycerin-treated, and in 3 of the saline-treated controls, all of which were shown to be hypersensitive during and up to the end of the experiment? In Table III are tabulated the animals from all three groups of Series II that showed the lesion under consideration. From examining this it is quite plain that the absence of hypersensitivity cannot be regarded as the explanation of the excessive growth of the bacilli.

Because O. T. contains a certain amount of glycerin, we gave this substance to one of the control groups of animals. Theoretically,

from the amount used to prepare the synthetic medium in these experiments there would be 20% glycerin in the final concentrated tuberculin. However, it is well known that much of this is used up by the tubercle bacilli (12), and some also evaporates when the tuberculin is concentrated. We felt that we were justified in using a 10% solution of glycerin to control the amount in the O. T., although we realized that the tuberculin probably did not contain exactly this concentration of glycerin. That the administration of glycerin might be a factor in permitting the bacilli to proliferate was suggested by experiments of Long and Vorwald (13). These investigators gave rats as much as 80 gm. of glycerol over a period of 168 days following infection and found that tubercle bacilli were more numerous in the lungs of the animals receiving glycerin than in the controls that received nothing. As was pointed out above, 28% of the animals that received glycerin in the present experiment developed the lesions teeming with bacilli; but the presence of the same lesion in 3 of the saline controls leads one to conclude that glycerin at least is not the only factor responsible for the occurrence of lesions of this type.

Willis and Woodruff (8) have emphasized that in their series of animals the lesion of this type did not appear before the 8th week, but was a constant finding from that time on, and they believe that its appearance is related to the length of time during which the treated animals remain alive. In our animals the great proliferation of acid-fast organisms appeared for the first time as early as the 6th week in both an O. T.- as well as in a glycerin-treated animal, and in the animals surviving longest it did not appear at all. The lesion was not present in 9 of the 12 O. T.-treated animals living 8 weeks, or longer. From these facts it seems safe to conclude that the duration of treatment is not the decisive factor in the development of the lesion.

It will be seen from Table III that 11 out of the 16 animals from the second series showing this lesion had been given 2 cc. of either O. T., glycerin, or saline, while 5 received only 1 cc. of these materials. The fact that the lesion occurred twice as frequently in the animals receiving the larger amounts suggests that the daily trauma may have had some influence on the occurrence of the lesion. In this connection it may be recalled that none of the controls in Series I received any injections, nor did the controls of Willis and Woodruff, and none of

these animals showed the great proliferation of bacilli. That the daily injections did affect the animals' well-being is clear from the fact that even those injected with saline lost weight, while during the same time uninjected controls did not.

What the predisposing factor is that permits the free growth of bacilli in certain of the animals cannot at present be stated with any assurance. Willis and Woodruff (8, suggested that the inanition and

TABLE III

WEEKS LIVED	ANIMAL NUMBER	MATERIAL PER DAY*	WEIGHT CHANGE	CONDITION AT DEATH†	TESTS FOR HYPERSENSITIVITY; DAYS BETWEEN TEST AND DEATH
		cc.	*per cent*		
6th	144	2 O.T.	−28	M	Neg. O.T. 16 and 2 days
	135	1 O.T.	−32	M	Neg. O.T. 16 and 2 days
	186	2 G.	−24	D	Pos. O.T. 16 and 2 days
	171	2 S.	−15	K	Pos. O.T. 18 and 4 days
7th	136	1 O.T.	−24	M	Neg. O.T. 22 and 8 days; neg. Bac. 2 days
	147	2 O.T.	−28	M	Neg. O.T. 28, 14, and 3 days; neg. Bac. 8 days
	178	2 G.	−29	K	Pos. O.T. 28, 14, and 3 days; pos. Bac. 8 days
8th	140	2 O.T.	−20	D	Neg. O.T. 34, 20, and 9 days; neg. Bac. 14 days
	189	1 G.	−20	M	Pos. O.T. 33, 19, and 8 days; pos. Bac. 13 days
	169	2 S.	−27	D	Pos. O.T. 31, 17, and 6 days; pos. Bac. 11 days
9th	192	2 G.	−20	M	Pos. O.T. 39, 25, and 14 days; pos. Bac. 19 days
	170	2 S.	−14	D	Pos. O.T. 41, 27, and 16 days; pos. Bac. 21 days
10th	150	2 O.T.	−12	D	Neg. O.T. 46, 32 and 21 days; neg. Bac. 26, 4 days
	146	2 O.T.	−11	M	Neg. O.T. 47, 33, and 22 days; neg. Bac. 27, 5 days
	179	1 G.	−34	M	Pos. O.T. 44, 30, and 19 days; pos. Bac. 24, 2 days
11th	182	1 G.	−22	D	Pos. O.T. 52, 38, and 27 days; pos. Bac. 32, 10 days

* O.T., Old Tuberculin; G., glycerin; S., Saline.

† M., moribund; D., died; K., killed.

emaciation of the tuberculin-treated animals might be responsible for the increased growth of bacilli but they thought it more likely that the loss of hypersensitivity was the important factor. Our control experiments do not support that conclusion. It is, however, true that many of the animals receiving daily injections become emaciated. It is striking that in spite of the abundance of phagocytic cells in the lesions, most of the bacilli appear to be extracellular. Phagocytosis

appears to be decidedly less active than even in normal animals. The free growth of bacilli appears to be the result of either a depression of natural resistance or an interference with the development of acquired resistance. At present it can be said only that the daily injections of O. T. or of glycerin, or of saline, tend to bring the body of certain guinea pigs into a condition that is peculiarly favorable for the growth of the bacilli, and that the presence or absence of hypersensitivity is not the determining factor.

That some other infection might also be present in the lungs of these animals was also considered. However, bacterial stains failed to reveal any other organism. Although cultures were not made, it seems unlikely that the enormous number of bacilli stained in the lesions might be some other kind of acid-fast organism such as the hay bacillus.

The greater proliferation of bacilli in the lungs than in the livers and spleens in the animals that showed this unusual growth of organisms, is likewise difficult to explain, for it is well known that in the guinea pig the spleen and liver are ordinarily more affected than the lungs. As was pointed out above, our findings did not agree with those of Willis and Woodruff (8) who had emphasized the fact that, although the spleens of animals showing the lesion teeming with bacilli also contained many organisms, there were practically no microscopic changes. Tuberculous lesions were present in the spleens of all our animals.

Another interesting question is the intense response of the hypersensitive control animals to tubercle bacilli introduced intracutaneously, while permitting the bacilli to grow in the pulmonary alveoli practically undisturbed. In the lungs in these animals the bacilli are certainly little, if at all, damaged by the body, and they proliferate to enormous numbers without exciting much tissue damage even in the hypersensitive animals. When fresh bacilli are introduced from without into the skin, however, a Koch phenomenon occurs. Why the bacilli that have proliferated in the body fail to excite as marked a reaction as those introduced from without, requires further investigation.

In human as well as in experimental tuberculous infection, antibody

has frequently been demonstrated in the serum. However, the exact significance of complement-fixing antibodies, agglutinins and precipitins, in relation to immunity in tuberculosis, is unknown at present. In experimental infections in guinea pigs, complement fixation has been described by several observers. It must be noted, however, that many different antigens have been used so that the results are in the main not comparable. Besredka and Manoukhine (14) noted fixation of complement as early as 4 days after infection. Klopstock (15) could not demonstrate this so early but found that the sera of his animals contained complement-binding antibodies later. Herrmann (16) has also demonstrated this phenomenon in guinea pig tuberculosis. The tuberculin reaction in relation to the complement-binding power was studied by Klopstock in experimental tuberculosis. He found that, although in most cases they more or less parallel each other, in some animals one or the other phenomenon may be absent. This agrees with a recent study of Freund and his associates (17) who found that rabbits lost their skin sensitivity to tuberculin towards the end of the course of experimental tuberculosis, yet still retained an undiminished complement-fixation titer. Higginbotham (7) found complement-fixation after desensitization in guinea pigs.

In the 44 sera examined for complement-fixation antibody we obtained positive results in 24 or 54% of the sera. The titer in these animals varied from rather weak reactions (1–3) to strong reactions (1–112). There was no constant correlation between the strength of antibody and the degree of tuberculosis. The only relation of importance seemed to be the duration of the infection, as the animals that lived longest tended to have higher titers than those coming to autopsy earlier.

The agglutination reaction has been extensively applied to tuberculous infection and most workers have felt that the technique was too faulty to use. However, recently Mudd (9) has described a new method which seems a decided improvement on previous ones. Using this technique in our experiments we obtained positive agglutination reactions in 37 out of the 44 sera tested (84%). Thus more positive reactions were obtained with this technique than with the complement-fixation procedure. As in the latter, no correlation existed be-

tween the character or extent of the lesions and the agglutinin titer. The complement-fixation and agglutinin titers more or less paralleled one another in each serum studied.

From this study of the antibodies only one point of interest emerges: the development of antibody in non-hypersensitive animals. But as was pointed out above, the significance of these antibodies as an index of the animal's immunity has yet to be demonstrated.

CONCLUSIONS

1. The lesions of animals infected with the tubercle bacillus, and prevented from becoming hypersensitive by treatment with O. T., show less caseation than those of control animals that develop hypersensitivity.

2. In some of the non-hypersensitive animals pulmonary lesions develop which are marked by an extraordinary proliferation of acid-fast bacilli. Since in the majority of the non-hypersensitive animals these lesions did not develop, and since the same lesions were found in some of the animals of the control groups shown to be hypersensitive, it is concluded that lack of hypersensitivity does not play a rôle in producing the lesions of this type.

I should like to express my appreciation to Dr. Arnold R. Rich for the advice and help he has given during the course of these experiments. I also wish to thank Dr. Harry Eagle for his generous aid in the complement-fixation determinations.

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PLATE 1

(Photographs by Mr. Milton Kougl)

FIGS. 1, 2, AND 3. Sites of intracutaneous injections of a suspension of live tubercle bacilli 48 hours before. Fig. 1. O.T. treated non-hypersensitive animal (No. 145) shows no reaction. Fig. 2. Glycerin-treated hypersensitive animal (No. 183) shows the Koch phenomenon with necrosis. Fig. 3. Saline-treated hypersensitive animal (No. 166) shows the Koch-phenomenon with necrosis.

FIG. 4. Low power view of pulmonary lesion in non-hypersensitive O.T.-treated animal (No. 147) to show alveoli packed with leucocytes and bacilli (H. and E.).

FIG. 5. High power view of above to show enormous numbers of acid-fast bacilli (carbol-fuchsin stain).

FIG. 6. Low power view of pulmonary lesion in glycerin-treated hypersensitive animal (No. 178) to show alveoli packed with leucocytes and bacilli (H. and E.).

FIG. 7. High power view of above to show the enormous numbers of acid-fast bacilli (carbol-fuchsin stain).

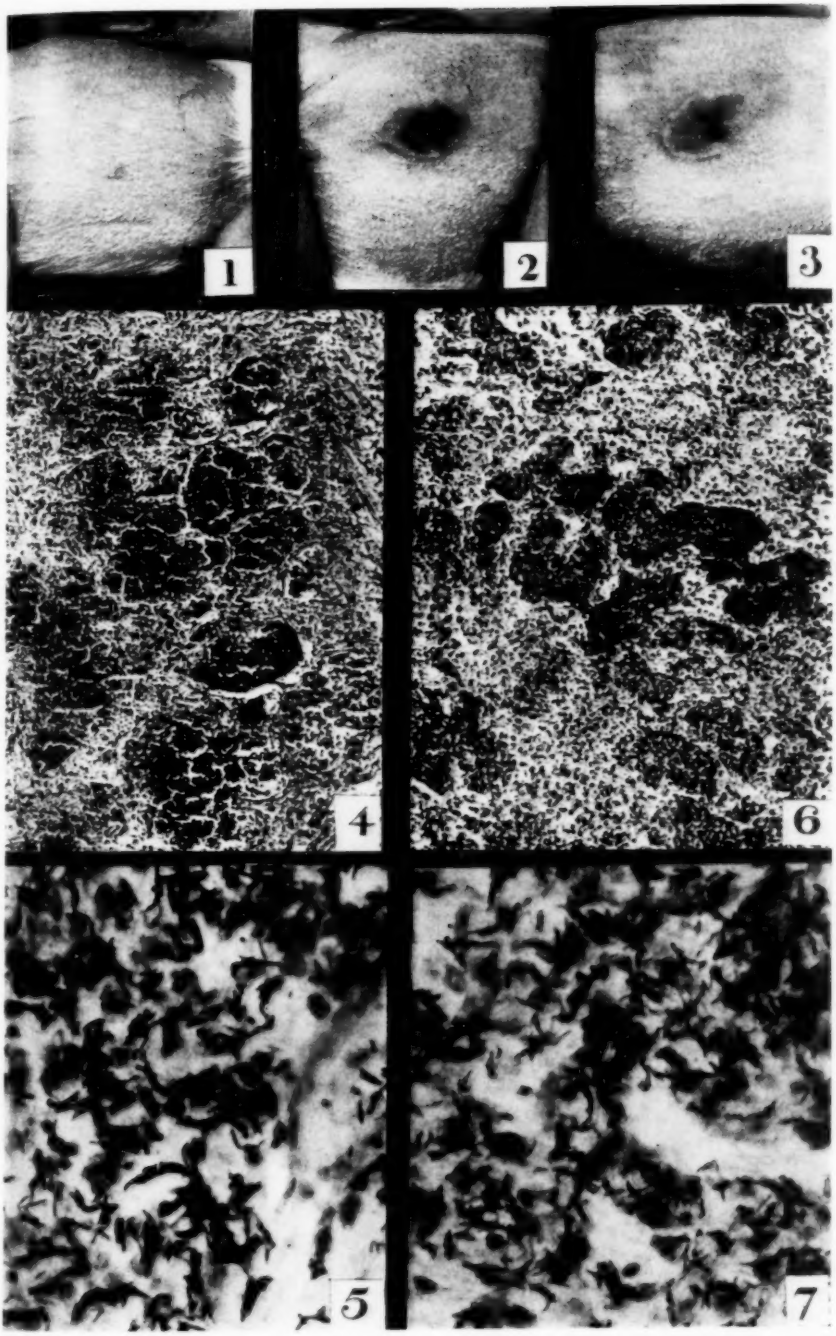

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PLATE 2

(Photographs by Mr. Milton Kougl)

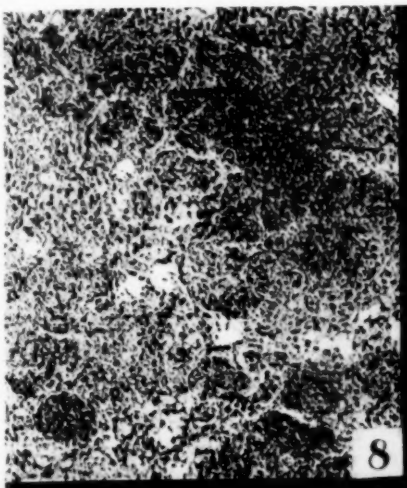
FIG. 8. Low power view of pulmonary lesion in saline treated hypersensitive animal (No. 169) to show alveoli packed with leucocytes and bacilli.

FIG. 9. High power view of above to show the enormous numbers of acid-fast bacilli (carbol-fuchsin stain).

FIG. 10. Tracheo-bronchial lymph nodes, lungs and spleen of O.T.-treated non-hypersensitive animal (No. 139) that has failed to show lesion containing enormous numbers of bacilli in the lungs and whose spleen shows marked involvement.

FIG. 11. Tracheo-bronchial lymph nodes, lungs and spleen of O.T.-treated non-hypersensitive animal (No. 146) that showed excessive proliferation of bacilli in lungs. The spleen is not much involved.

FIG. 12. Tracheo-bronchial lymph nodes, lungs and spleen of glycerin-treated hypersensitive animal that showed marked involvement of the lungs with an excessive proliferation of bacilli.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ON THE SPIROCHETICIDAL ACTION OF BISMUTH COMPOUNDS ON PATHOGENIC SPIROCHETA PALLIDA IN VITRO

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Although bismuth compounds are widely used as adjuncts to arsphenamine in the treatment of syphilis, comparatively little is known as to their mode of action. Most of the experimental work deals with the rates of absorption and excretion and with the relative therapeutic efficacy of various soluble and insoluble bismuth preparations. Several theories have been suggested as to how this therapeutic action is effected; but the supporting experimental data are conflicting and inconclusive.

Giemsa (1) reported that a proprietary preparation of bismuth ("Bismutyl") in a dilution containing 0.035 per cent of bismuth metal, did not kill pathogenic *Sp. pallida in vitro* in ½ hour at 37°C. Similarly, Levaditi and his coworkers (2) (3) found that bismuth compounds *per se* had no demonstrable effect *in vitro* on trypanosomes or on *Sp. gallinarum*. However, if the bismuth compound was first incubated with bits of tissue or with tissue extracts, it became markedly trypanocidal and spirocheticidal *in vitro*. They concluded that an active Bi-protein complex had been formed which they termed "Bismoxyl." They subsequently suggested (4) (5) that glutathione is primarily responsible for the formation of this hypothetical bismuth-tissue complex. "Bismoxyl" had no therapeutic effect in trypanosomiasis, but proved highly active in their hands in the treatment of syphilitic rabbits. They concluded that the formation of "Bismoxyl" *in vivo* is primarily responsible for the therapeutic activity of bismuth compounds.

[1] Aided by a grant from the Committee on Research in Syphilis, Inc.

Kolle and Evers (6), on the other hand, have presented evidence that bismuth is not actively spirocheticidal even *in vivo*, and merely inhibits the growth of *Sp. pallida*. Rabbits in which they placed an intramuscular or subcutaneous depot of an unspecified insoluble bismuth compound suspended in oil developed no chancre when subsequently inoculated with *Sp. pallida*. Despite the absence of a primary lesion, infection could be proved in a large proportion of cases by lymph node transfer. Moreover, when the bismuth depot was removed, chancres often developed at the site of the original inoculation. These experiments suggested that bismuth inhibits the development of spirochetes, and is not actively spirocheticidal. Levaditi and his coworkers (7) (8) have criticized these observations on the ground that Kolle and Evers used inadequate amounts of bismuth. With larger bismuth depots, or with smaller amounts of more soluble preparations, Levaditi obtained complete protection against syphilitic infection.

In other papers (9) (10) (11), Levaditi and his coworkers stressed the excessively minute concentrations of bismuth which can be recovered from the organs of syphilitic rabbits treated by the intramuscular injection of insoluble bismuth compounds. Thus, only 2–10 gamma of bismuth per gram of dry tissue could be recovered from the testicular chancres of rabbits treated with 0.1 gm. per kg. of a preparation containing 64 per cent bismuth. They therefore suggested that bismuth compounds are not directly spirocheticidal, but function as catalysts for an unspecified lytic mechanism.

In summary, it has been suggested (1) that bismuth functions as a catalyst for spirochetolysis *in vitro*; (2) that it is inactive *per se*, but combines with tissue derivatives to form actively spirocheticidal compounds; (3) that it merely inhibits the multiplication of the spirochetes.

The work of Levaditi and of Giemsa apparently excluded the possibility that bismuth compounds as such may be actively spirocheticidal, and that their therapeutic action may be primarily due to such activity; at least, that possibility has not been seriously considered.[2] Later investigators have apparently overlooked the fact that although

[2] The finding of Kadisch (12) that bismuth compounds killed cultures of *Sp. pallida in vitro* is perhaps not relevant to the present discussion, in view of the doubt as to the validity of identifying such cultures as *Sp. pallida*.

Giemsa found 0.035 per cent bismuth to be inactive, twice that concentration did kill pathogenic *Sp. pallida* obtained from rabbit chancres in 30 minutes at 37° (1).

It has been recently shown (13) that, contrary to previous reports, arsphenamine, neoarsphenamine and silver arsphenamine, immobilize and kill *Sp. pallida in vitro*. The available evidence strongly suggested that this spirocheticidal action was either a function of the arsphenamines *per se*, or of their oxidation products, but was not due to the formation of a tissue-arsphenamine complex. In view of these findings, it seemed worthwhile to reëxamine the possibility that bismuth compounds, like the arsphenamines, might be directly spirocheticidal *in vitro*, and that it might accordingly be unnecessary to assume either a catalytic function for bismuth or the formation of a hypothetical bismuth-tissue complex.

As will be shown in the present paper, this was found to be the case. All the bismuth compounds which could be tested were found to immobilize *Sp. pallida in vitro*. Moreover, the immobilized organisms were shown to be non-infectious for rabbits and presumably dead. The possibility must therefore be considered that the therapeutic action of bismuth in syphilis depends in part, and perhaps primarily, on its direct spirocheticidal action.

METHODS

Unless otherwise stated, the Nichols strain of *Sp. pallida* was used throughout. As described in a preceding paper (13), rabbits were inoculated intratesticularly, and the testes were removed during the early stage of acute inflammatory edema. Each testis was finely minced, and the mash was ground in a mortar with 5 cc. of rabbit serum and 5 cc. of 0.85 per cent salt solution. The mixture was then centrifuged for ten minutes at 1500 r.p.m. to remove tissue particles and most of the formed cells. The clear or opalescent supernatant fluid used in the experiment usually contained more than ten organisms per microscopic field (dark field illumination, 900 × magnification).

In order to determine the effect on motility, measured amounts of freshly prepared dilutions of the various bismuth compounds were brought up to 0.4 cc. with 0.85 cc. per cent NaCl, and 0.4 cc. of the

spirochetal suspension was then added. The tubes were then placed in a Brown anaerobe jar at room temperature (24° to 27° C.). Two to four hours later, the tubes were removed from the anaerobe jar, and the proportion of motile organisms was determined by direct count. At least fifty, and usually one hundred, organisms were counted for each determination.

EXPERIMENTAL

For obvious reasons, only water-soluble bismuth compounds could be tested quantitatively for antispirochetal activity. Individual experiments with six soluble preparations available commercially are summarized in Table I. Each horizontal row in that table represents an individual experiment, carried out at a different time and with a different chancre emulsion, and is illustrative of many similar experiments. Since they were not performed simultaneously, they are not quantitatively comparable.

It is evident that all the preparations used immobilized *Sp. pallida in vitro*. Several of the preparations formed heavy precipitates when added to serum or tissue extracts; as would be expected, their activity was correspondingly impaired. These compounds excluded, under the particular experimental conditions of Table I, bismuth compounds in concentrations of approximately 1:20,000 to 1:60,000 (calculated as bismuth metal) exerted a significant antispirochetal action in two to four hours at room temperature.

As was true of the arsphenamines, when the same drug was tested repeatedly, with different chancre emulsions and varying periods of incubation, widely varying results were obtained for the minimal effective concentration (cf. fourth column of Table III).

An attempt was now made to determine the antispirochetal activity of the various bismuth compounds relative to that of "arsenoxide" (the hydrochloride of m-amino-p-hydroxyphenylarsenoxide), by simultaneous determinations of the minimal effective concentrations of the bismuth and arsenic compounds, using the same chancre emulsion under the same experimental conditions. The results are summarized in Tables II and III. Table III is a condensation of four Tables like Table II. Compared mole for mole, these bismuth compounds were approximately 1/4 to 1/28 as active as "arsenoxide".

No explanation can as yet be given for the widely varying results obtained on repeated tests with the same compound.

As shown in Table IV, organisms immobilized by bismuth compounds were non-infectious for rabbits and presumably dead. Rabbits inoculated intratesticularly with organisms immobilized by

TABLE I

The Antispirochetal Action of Six Bismuth Compounds on Sp. pallida in vitro

PREPARATION NUMBER	BISMUTH CONTENT	DILUTION OF BISMUTH PREPARATION	TIME OF EXPOSURE	BISMUTH SOLUTION, CC.					CONTROL	APPROXIMATE CONCENTRATION OF BISMUTH (AS METAL) † WHICH IMMOBILIZES 50% ‡ OF THE ORGANISMS
				0.4	0.2	0.1	0.05	0.025	0	
				0.85 % NaCl, cc.						
				0	0.2	0.3	0.35	0.375	0.4	
				Chancre emulsion, cc.						
				0.4	0.4	0.4	0.4	0.4	0.4	
				Proportion of motile organisms after incubation at room temperature, %						
	per cent		*hours*							
1	41	1:1000	4	0	24	37	60	71	83	1:25,000
2	3.3	1:40	3	0	0	15	37		85	1:20,000
3	2.3	1:100	4	0	0	64	76		82	1:25,000
4	2.5	1:100	2	0	0	21	54		82	1:60,000
5	2.0*	1:30	3	0 ppt.	44 ppt.	ppt.	62		80	1:6000
6	75§	1:250	3	20 ppt.	32 ppt.		56		80	1:2500

* Approximate only.

† Effective concentration of preparation × proportion of bismuth in preparation. Thus, in the first horizontal row, a final concentration of 1:10,000 of preparation #1 had a significant effect: this represents a bismuth concentration of 1:10,000 × 0.41 = 1:25,000.

‡ In this and all following tables the immobilization of 50% of the organisms refers to 50% of those actively motile in the control tube at the end of the experiment. In the first horizontal row of this table the 50% end-point is half of 83, or 41 organisms; in the second experiment of Table II it is half of 92, or 46 organisms, etc.

§ Manufacturer's figure.

bismuth compounds did not develop primary lesions, and lymph node transfers from those animals into normal rabbits were similarly negative (Table IV). The minute amounts of bismuth present in the mixture did not act by preventing infection *in vivo*. This was shown by the fact that even when ten times the minimal immobilizing

TABLE II

*Antispirochetal Action of a Single Bismuth Preparation Compared to that of "Arsenoxide"**

Bi Preparation (#3 of Table I) = 2.3% Bi. Arsenoxide = 31.9% As

PREPARATION	DILUTION USED IN EXPERIMENT	TIME OF EXPOSURE	AMOUNT OF SOLUTION, CC.							CONTROL	APPROXIMATE DILUTION OF DRUG WHICH IMMOBILIZED 50% OF ORGANISMS†	EFFECTIVE CONCENTRATION OF Bi OR As		RELATIVE ACTIVITY OF Bi/As EXPRESSED AS RATIO OF EFFECTIVE MOLAR CONCENTRATION
			0.4	0.28	0.2	0.14	0.1	0.07	0.05	0		Absolute concentration‡	Molar concentration§	
			0.85% NaCl, cc.											
			0	0.12	0.2	0.26	0.3	0.33	0.35	0.4				
			Chancre emulsion, cc.											
			0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4				
			Proportion of motile organisms at end of incubation period, %											
		hours												
Bismuth	1:100	4	0		0		64		76	84	1:600	1:26,000	1:5450	1:5.3
Arsenoxide	1:40,000		4		56		80				1:123,000	1:386,000	1:29,000	
Bismuth	1:200	5	0	13	58					92	1:700	1:30,400	1:6350	1:6.6
Arsenoxide	1:40,000		0	0	33	70					1:178,000	1:560,000	1:42,000	
Bismuth	1:500	3	5	22	74		60			89	1:1400	1:60,800	1:12,700	1:8.1
Arsenoxide	1:100,000		0	8	17	31					1:440,000	1:1,375,000	1:103,000	
Bismuth	1:200	4	10	39	66					84	1:600	1:26,000	1:5450	1:6.9
Arsenoxide	1:50,000		8	36	55						1:160,000	1:500,000	1:37,500	

* Hydrochloride of m-amino-p-hydroxyphenylarsenoxide.

† Cf. Note ‡, Table I.

‡ Effective concentration of drug × proportion of Bi (As) = effective concentration of Bi (As).

§ $\frac{\text{Effective concentration of Bi (gms. per liter)}}{\text{atomic weight (209)}}$ = molar concentration of Bi.

Similarly for As (atomic weight 75).

concentration of bismuth was added to live spirochetes, and the mixture immediately inoculated into rabbits, the animals regularly developed typical dark field positive testicular chancres (Table V).

TABLE III

*The Antispirochetal Action in vitro of Four Bismuth Compounds Compared to that of "Arsenoxide"**

PREPARATION NUMBER†	BISMUTH CONTENT, %	TIME OF INCUBATION WITH SPIROCHETAL SUSPENSION	APPROXIMATE DILUTION OF DRUG WHICH IMMOBILIZES 50% OF ORGANISMS UNDER CONDITIONS OF EXPERIMENT	APPROXIMATE DILUTION OF "ARSENOXIDE" WHICH IMMOBILIZES 50% OF ORGANISMS UNDER SAME CONDITIONS	RELATIVE ACTIVITY OF BISMUTH COMPOUND AND "ARSENOXIDE"	
					Per gm. Bi and As‡	Per mole Bi and As§
		hours				
		3	1:5750	1:143,000	1:32	1:11.5
		3	1:5000	1:82,000	1:31.2	1:11.2
1	41	3	1:8000	1:160,000	1:25.8	1:9.3
		2½	1:5000	1:150,000	1:37.8	1:13.6
		2	1:8000	1:3,200,000	1:41.6	1:14.9
		3	1:1325	1:142,500	1:11.1	1:3.87
		3	1:800	1:84,000	1:10.95	1:3.95
2	3.3	4	1:1100	1:123,000	1:11.7	1:4.1
		3	1:1000	1:160,000	1:16.7	1:6
		2	1:700	1:296,000	1:44.2	1:15.8
		2½	1:200	1:150,000	1:78	1:28.1
		4	1:600	1:123,000	1:14.8	1:5.3
		5	1:700	1:178,000	1:18.4	1:6.6
3	2.3	3	1:1400	1:440,000	1:22.5	1:8.15
		3	1:600	1:160,000	1:19.2	1:6.9
		2½	1:600	1:150,000	1:18.0	1:6.5
		1½	1:1125	1:250,000	1:17.4	1:6.3
4	2.5	3	1:700	1:160,000	1:17.8	1:6.4
		2½	1:200	1:150,000	1:64.5	1:23

* The hydrochloride of m-amino-p-hydroxyphenylarsenoxide, containing 31.9% arsenic.

† Numbers correspond to those of Table I.

‡ Thus, in the first horizontal row, a 1:5750 dilution of bismuth compound #1 represents a 1:14,000 dilution of bismuth; a 1:143,000 dilution of arsenoxide represents a 1:448,000 dilution of arsenic; and since these two dilutions were equally effective, arsenic was thirty-two times as active as bismuth, gram for gram.

§ Atomic weight of bismuth = 209; atomic weight of arsenic = 75; molar ratio of bismuth to arsenic therefore is 209/75 = 1/0.36 × weight ratio.

TABLE IV

Showing that Organisms Immobilized by Bismuth Compounds in vitro are Non-infectious for Rabbits and Presumably Dead

Preparation 1*											CONTROL	
Final concentration of bismuth in experimental mixture	1:2437		1:4875		1:9750		1:19500		1:39000		0	
Proportion of motile organisms after four hours at 26°C., %	0		0		0		8		64		68	
Result of intratesticular inoculation of experimental mixture into rabbits† ‡	0	0	0	0	0	0	+		+		+	+
Result of lymph node transfer from inoculated rabbits into normal rabbit†	0	0	0	0	0	0	+		+		+	+

Preparation 2							CONTROL	
Final concentration of bismuth in experimental mixture	1:3000	1:6100	1:12200	1:24400	1:48800		0	
Proportion of motile organisms after four hours at 25°C., %	0	0	20	40	86		88	
Result of intratesticular inoculation of experimental mixture into rabbits†	−§	0	0	+	+		+	+
Result of lymph node transfer from inoculated rabbits into normal rabbit†	+	0	+	+	Not done		+	+

Preparation 3										CONTROL	
Final concentration of bismuth in experimental mixture	1:4400		1:8800		1:17600		1:35200		1:70400	0	
Proportion of motile organisms after four hours at 26°C., %	0		0		4		32		66	68	
Results of intratesticular inoculation of experimental mixture into rabbits†	−§	0	+	0	+		+	+	+	+	+
Results of lymph node transfer from inoculated rabbits into normal rabbit†	0	0	0	0	Not done		+	+	Not done	+	+

* These numbers correspond to those of Table I.

† Examined frequently over a ten-week period for development of chancre: testes finally removed, emulsified, and examined under dark field.

‡ In this and the following table, each 0 or + mark indicates a single animal.

§ Animal died before the results of the testicular inoculation could be established. Node transfer from this to another rabbit was, however, feasible, as indicated.

It is to be noted (Table IV, Preparation 3) that in "borderline" concentrations of bismuth, which barely sufficed or just failed to immobilize all the organisms, variable results were sometimes ob-

TABLE V

Showing that the Non-infectiousness of Bismuth-treated Spirochetes (Table IV) is not Due to an in vivo Action of the Bismuth

Varying amounts of bismuth, exceeding tenfold those used in the experiments of Table IV, were mixed with a chancre extract, and the mixture immediately inoculated into rabbits intratesticularly. The minimum concentrations necessary to prevent infection were many times greater than those which sufficed to render the organisms non-infectious after incubation. The spirocheticidal action was therefore primarily an *in vitro* effect.

Preparation 1*

			CONTROL	
Final concentration of bismuth in experimental mixture	1:245	1:975	0	
Results of intratesticular inoculation of experimental mixture	0	+	+	+
Results of lymph node transfer from inoculated animals into normal rabbits	0	+	+	0

Preparation 2

Final concentration of bismuth in experimental mixture	1:305	1:1220
Results of intratesticular inoculation of experimental mixture	0	+
Results of lymph node transfer from inoculated animals into normal rabbits	0	+

Preparation 3

					CONTROL	
Final concentration of bismuth in experimental mixture	1:440		1:880	1:1760	0	
Results of intratesticular inoculation of experimental mixture	0	+	+	+	+	+

* Numbers correspond to those of Table I.

tained on animal inoculation. Similar variable results had been previously obtained with borderline concentrations of the arsphenamines (13).

As shown in Table VI, the antispirochetal action of bismuth just

described was not peculiar to the Nichols strain, but was also observed with three strains of *Sp. pallida* freshly isolated from cases of early human syphilis with open lesions, and tested experimentally after one or two rabbit passages. These were also immobilized and rendered non-infectious.

In view of the theory of Levaditi that bismuth compounds are not spirocheticidal *per se*, but become so only after activation by tissue, it became pertinent to inquire to what extent tissue extractives neces-

TABLE VI

Showing that Strains of S. pallida Freshly Isolated from Human Lesions and Tested After One or Two Rabbit Passages Are Also Susceptible to Bismuth Compounds in vitro

SPIROCHETE STRAIN NUMBER	BISMUTH PREPARATION NUMBER*	BISMUTH CONTENT	DILUTION OF PREPARATION USED IN EXPERIMENT	TIME OF INCUBATION AT ROOM TEMPERATURE	BISMUTH SOLUTION, %					CONTROL	APPROXIMATE CONCENTRATION OF BISMUTH (As METAL) WHICH IMMOBILIZED 50% OF ORGANISMS
					0.4	0.2	0.1	0.05	0.025	0	
					0.85% NaCl, cc.						
					0	0.2	0.3	0.35	0.375	0	
					Chancre mash, cc.						
					0.4	0.4	0.4	0.4	0.4	0.4	
					Proportion of motile organisms after incubation period, %						
		per cent		*hours*							
A†	1	41	1:1000	$2\frac{3}{4}$	0	0	26	68	80	82	1:25,000
B†	3	2.3	1:400	5	0	13	58			92	1:100,000
C†	1	41	1:1000	4	0	24	41			84	1:20,000
	2	3.3	1:200		0	14	61				1:34,000
	3	2.3	1:200		10	39	66				1:34,000
	4	2.5	1:200		6	51	80				1:34,000

* Numbers correspond to those of Table I.

† Strains A and B tested after two rabbit passages; strain C tested on first rabbit passage.

sarily present in the chancre mash were responsible for the observed antispirochetal action. Accordingly, a chancre mash was prepared in the usual manner, by grinding a chancre testis with 10 cc. of 50 per cent rabbit serum, and portions of the centrifuged extract were diluted (a) with 50 per cent serum, and (b) with concentrated extracts of normal testes, prepared by emulsifying normal testes in 5 cc. of the diluted rabbit serum. A series of spirochete suspensions were thus obtained, varying at least eighteenfold in the concentration of tissue

extractives. Each of these suspensions was now added to varying concentrations of a bismuth preparation, and the proportion of motile organisms determined in the usual manner after three hours in an anaerobe jar. One of two experiments, both with qualitatively

TABLE VII

The Inhibitory Effect of Tissue Extractives on the in vitro Antispirochetal Action of a Bismuth Compound (#3 of Table I)

TYPE OF SPIROCHETE EMULSION USED	DILUTION OF BISMUTH METAL IN FINAL EXPERIMENTAL MIXTURE										CONTROL	APPROXIMATE CONCENTRATION OF BISMUTH NECESSARY TO IMMOBILIZE 50% OF ORGANISMS
	600	1200	2400	4800	9600	19,200	38,400	76,800	153,600	307,200	0	
	Proportion of motile organisms after three hours at room temperature, %											
Stock suspension diluted with three volumes of concentrated testis extract	0	5	36								83	1:2500
Stock suspension diluted with equal volume of concentrated testis extract	0	0	27	54							85	1:3200
Stock suspension diluted with half volume normal testis extract	0	0	0	12	33						87	1:10,000
Chancre emulsified in 10 cc. 50% serum stock suspension	0	0	0	0	15	37					85	1:20,000
Stock suspension diluted with equal volume of 50% rabbit serum	0	0	0	0	0	0	19	44			88	1:76,800
Stock suspension diluted with three volumes of 50% rabbit serum	0	0	0	0	0	0	0	5	31	68	83	1:200,000

similar results, is summarized in Table VII. As is there shown, the tissue derivatives markedly inhibited rather than accentuated the antispirochetal action. When the chancre mash was diluted with a concentrated tissue extract, the antispirochetal action was correspondingly decreased. Conversely, when the tissue derivatives were

decreased by diluting the chancre extract with diluted serum, the antispirochetal action was correspondingly enhanced. At the two extremes there was an eightyfold difference in antispirochetal activity, apparently due solely to the varying concentrations of tissue extractives.

It is to be noted further that Levaditi and his coworkers, in their studies of "Bismoxyl", found that the activity of this hypothetical bismuth-tissue complex was largely independent of the bismuth concentration (2), but rather depended largely on the tissue concentration. In their experiments, the therapeutic activity of the mixture increased directly with an increase in the tissue extractive until the latter reached an optimum concentration, beyond which no change was observed (3). The present *in vitro* studies with *Sp. pallida* clearly demonstrate that the antispirochetal activity of a bismuth-tissue mixture increases directly and regularly with the concentration of bismuth, while the effect of tissue extractives is regularly one of inhibition. The present experiments thus fail to support the thesis that bismuth is activated by tissue extractives: as in the case of the arsphenamines, the tissue derivatives markedly inhibit rather than accentuate the direct action of bismuth compounds on *Sp. pallida in vitro*.

SUMMARY AND DISCUSSION

It would appear from the experiments here described that bismuth compounds kill pathogenic *Sp. pallida in vitro*. The organisms are immobilized, become non-infectious for rabbits, and are presumably dead. This effect was not peculiar to the Nichols strain, which has been passed through rabbits for over twenty-five years, but was also observed with strains freshly isolated from human cases of syphilis and tested after one or two rabbit passages. There was no indication that tissue derivatives contributed in any way to the antispirochetal action of bismuth; on the contrary, as in the case of the arsphenamines, tissue extractives regularly and markedly inhibited this activity. As much as an eighty-fold decrease in the apparent efficacy of a bismuth preparation could be produced by increasing the concentration of tissue extractives.

Two of six soluble bismuth preparations commercially available formed precipitates on addition to serum or tissue extracts; their

activity *in vitro* was correspondingly impaired. The remaining four preparations were approximately 1/4 to 1/28 as active as "arsenoxide", the bismuth and arsenic being compared on a molar basis in each instance. The possibility must therefore be considered that bismuth compounds act neither as catalysts, inhibitors, nor precursor substances, but that the therapeutic action of bismuth in syphilis depends in part, and perhaps primarily, on its direct spirocheticidal action on *Sp. pallida*.

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THE ABSORPTION AND EXCRETION OF CERTAIN SULFANILAMIDE DERIVATIVES[1]

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Considerable information is now available in regard to the absorption and excretion of sulfanilamide (1, 2, 3, 4). The acetyl-derivative, to which sulfanilamide is partly changed in man and other mammals, is much less readily absorbed when given in large doses by mouth (5). This may be due to its lesser solubility in water. In view of the differences in absorption found for sulfanilamide and acetyl-sulfanilamide, we have compared the absorption and excretion of sulfanilamide with two of its derivatives, one much more soluble in water, and the other almost insoluble.

Dogs were used for the experiments, due to the fact that the dog does not conjugate sulfanilamide or these derivatives, and that sulfanilamide appears to be absorbed and excreted in the dog in a way quite similar to that in man. Monoethanol-sulfanilamide ($NH_2C_6H_4SO_2NHC_2H_4OH$) soluble to about 4.4 per cent in water at room temperature was used as a more soluble compound; disulfanilamide ($NH_2C_6H_4SO_2NHC_6H_4SO_2NH_2$) soluble to about 0.01 per cent in water at room temperature as a very difficultly soluble compound; and sulfanilamide, soluble to about 0.8 per cent at room temperature, as a standard for comparison.[3] The drugs were administered by mouth

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[2] Drs. Cutting and Cover are Fellows in Experimental Therapeutics and Medicine.

[3] We wish to thank Dr. Perrin H. Long for the supply of the ethanol-sulfanilamide which was furnished by Dr. Roger Adams of the University of Illinois; Dr. O. W. Barlow of the Winthrop Chemical Company for the disulfanilamide, and Dr. K. K. Chen of Eli Lilly and Company for the sulfanilamide used in this investigation.

in gelatin capsules. In some of the experiments water was given by stomach tube in amounts of 15 cc. per kilo at 0, and 5 cc. per kilo at 2, 4, and 6 hours. Urine was obtained by catheter. Analyses of urine, blood and spinal fluid were made by the method described (6), with the exception that disulfanilamide determinations in blood were made by our original alcohol method (2). In many later experiments dilutions of blood of 1:50 were used and determinations made with a photoelectric colorimeter. Four female dogs were used, F, 6.5 kgm., K, 7.5 kgm., D, 22.5 kgm. and G, 9.5 kgm.

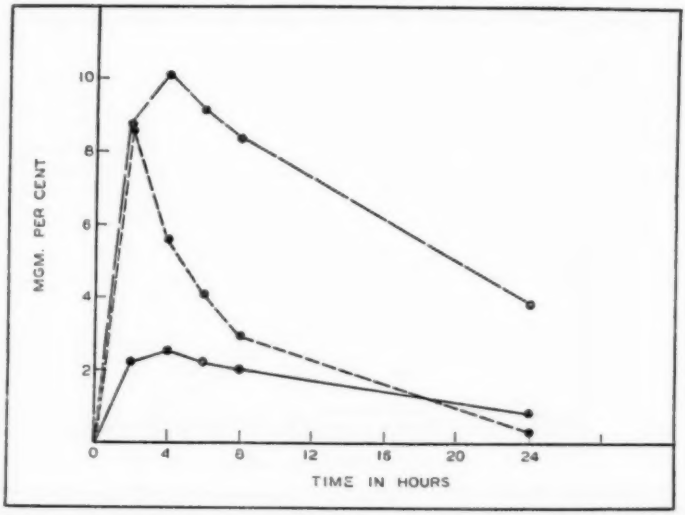

FIG. 1. Average blood concentration curves for 4 dogs after administration of 0.1 gm. per kilogram of sulfanilamide (—— ——), of ethanolsulfanilamide (----------), of disulfanilamide (————). The drugs were given in gelatin capsules by mouth.

A dose of 0.1 gram per kgm. of each of the drugs was given to each dog. The data obtained from analyses of samples of blood and urine collected at various intervals allow an estimation of the relative absorption and excretion rates of the three drugs. Figure 1 gives composite curves of the average values obtained for 4 dogs of blood concentrations at various times after ingestion of the drugs. Although some variations occurred in the different animals, the relative course

of the blood values for each drug was the same in all. A second series of experiments was done in which 1.0 gram per kgm. of each compound was administered to each dog. Figure 2 gives the composite curves of the average values for the four animals.

These blood curves, of course, depend on both the rates of absorption and excretion of the drugs. In table 1 are given the averages of

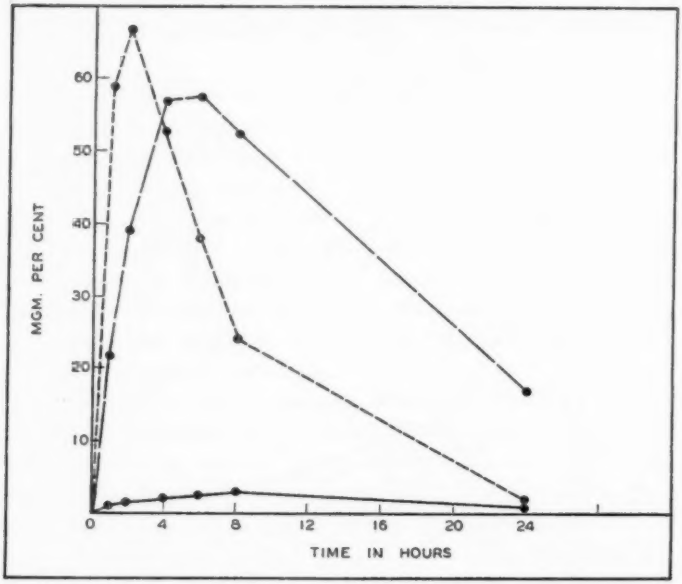

FIG. 2. Average blood concentration curves for 4 dogs after administration of 1.0 gm. per kilogram sulfanilamide (—— ——), of ethanolsulfanilamide (----------), of disulfanilamide (————). The drugs were given in gelatin capsules by mouth.

the percentage excretion for the four dogs of the drugs for 2, 4, and 8 hours both for the small and for the large dose. These figures taken in conjunction with the blood curves indicate that, with the small dose, disulfanilamide is more slowly absorbed but more rapidly excreted than sulfanilamide; ethanol-sulfanilamide is both absorbed and excreted much more rapidly than sulfanilamide. Confirmation of differences in excretion is obtained by calculating from the data

the renal clearances of the three substances for the small dose. These are given in table 2 calculated in cc. per minute. They are averages of the two or three periods of an experiment where a calculation of the clearance is possible. The ratios in the last two columns show that the sulfanilamide clearance is about one-fourth of that of each of the other compounds.[4] It should be pointed out that whole blood and not plasma concentrations have been used in calculating these clearances.

TABLE 1

Urinary excretion of sulfanilamide derivatives in percentage of amount ingested

	2 HOURS		4 HOURS		8 HOURS	
Dose, gm./kgm.	0.1	1.0	0.1	1.0	0.1	1.0
Sulfanilamide	5.5	3.8	13.5	12.5	26.0	21.8
Ethanol-sulfanilamide	17.2	16.0	44.0	35.2	66.0	60.8
Di-sulfanilamide	3.8	0.3	11.0	0.6	25.0	2.4

TABLE 2

Clearances of sulfanilamide derivatives

DOG	CLEARANCE OF			RATIO	
	S	Di-S	EtS	Di-S/S	Et-S/S
F	3.9	17.0	20.7	4.3	5.3
K	5.4	22.0	20.1	4.1	3.7
D	14.8	53.0	52.0	3.6	3.5
G	7.1	29.3	25.8	4.1	3.6

When the data for the larger dose are examined, it is seen that absorption is little impaired for sulfanilamide and ethanol-sulfanilamide, being possibly somewhat slower for the former. Disulfanilamide, on the other hand, is very poorly absorbed; the blood curve and absolute amounts excreted are little different from what is found with one-tenth the dose.

In all of the above experiments, the drugs were given by mouth in gelatin capsules. In some later experiments with sulfanilamide it

[4] The fact that the sulfanilamide clearance is about one-fourth that of creatinine in the dog suggests that the ethanol-sulfanilamide clearance may prove to be a measure of glomerular filtration rate.

was found that much more rapid absorption occurred if the compound was administered in solution on an empty stomach. In figure 3 are shown data from one dog on blood concentrations after giving a dose of sulfanilamide in a gelatin capsule and on another occasion the same dose in solution. Even when 25 cc. of water per kilogram was given immediately after the capsule, the absorption was essentially the same as without water. If absorption does not occur from the stom-

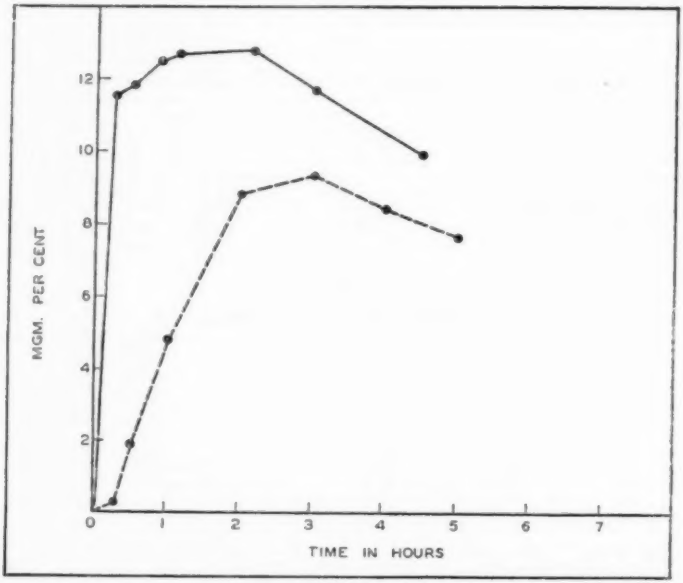

FIG. 3. Blood concentration curve after administration of 0.1 gm. per kilogram of sulfanilamide to a fasting dog on two occasions. Upper curve-drug given in solution by mouth, lower curve-drug given in gelatin capsule by mouth.

ach but occurs very rapidly from the intestine, the above facts would be explained.

Consequently, experiments on absorption from the stomach and intestine separately were done on dogs anesthetized with phenobarbital sodium. An incision was made in the abdomen, and the stomach ligated at the pylorus. After closing the abdominal wound, a solution of sulfanilamide was introduced into the stomach. During

the next two hours, samples of blood were taken and analyzed for sulfanilamide. The stomach was now washed out, and a solution of sulfanilamide introduced into the intestine. Blood samples were again taken during the course of the next two hours and analyzed for sulfanilamide. The results show that when given *in solution*, the sulfanilamide is absorbed in very small amounts from the stomach,

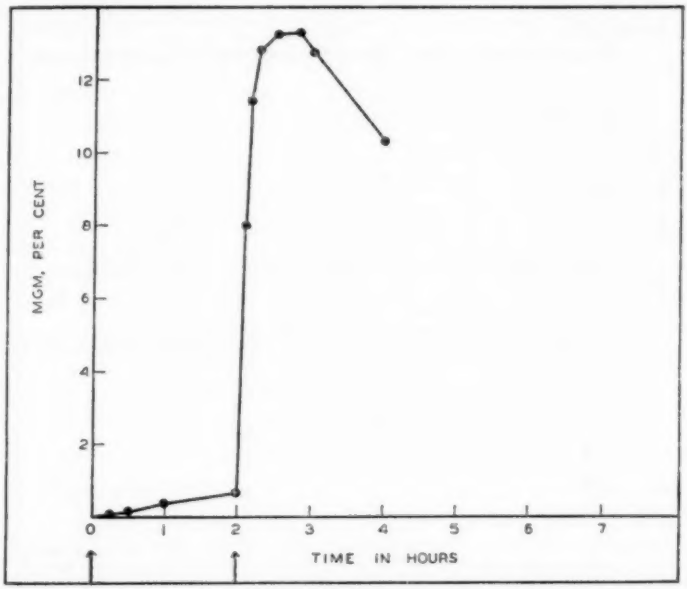

FIG. 4. Dog 11.1 kgm. anesthetized with 130 mgm. per kilogram of phenobarbital sodium. Pylorus tied and stomach washed with water. At first arrow, 0.1 gm. per kilogram of sulfanilamide in solution placed in stomach; at second arrow, stomach washed and similar amount of sulfanilamide in solution injected into the duodenum.

but is absorbed extremely rapidly from the intestine. The results of one experiment are shown in figure 4.

The fact that sulfanilamide passes readily into the cerebrospinal fluid and attains a concentration only slightly lower than that in the blood (2, 7) does not indicate that other compounds used as substitutes will act in a similar manner. In fact, Jauerneck and Gueffroy (8)

state that "prontosil soluble"[5] passes only very slowly and in small quantities into the spinal fluid of patients with meningitis. Millett

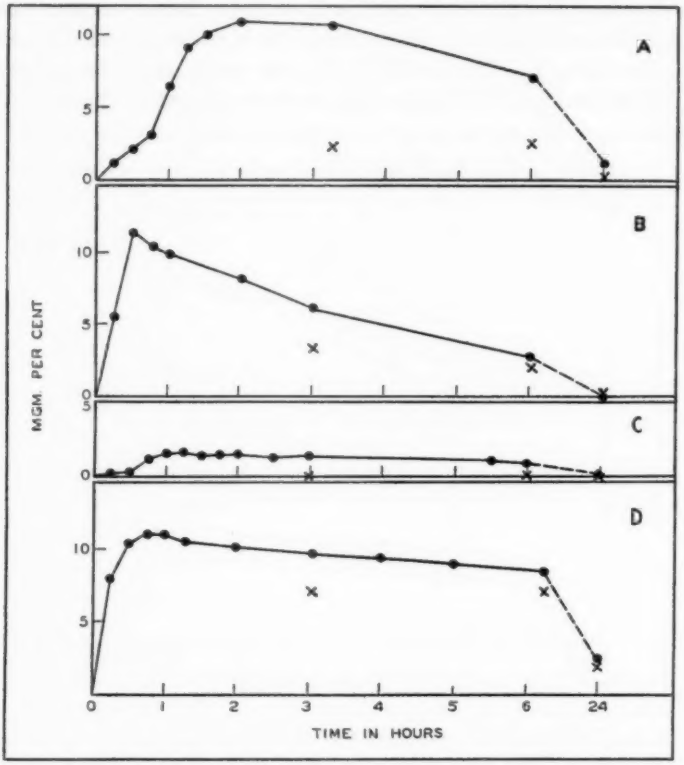

FIG. 5. The passage of sulfanilamide substitutes into cerebrospinal fluid. Dots (. . . .) represent concentrations of drugs in blood. Crosses (× × ×) represent the concentration of drugs in cerebrospinal fluid. (*A*) 0.1 gm. per kilogram of acetyl-sulfanilamide in capsule given by mouth; (*B*) 0.1 gm. per kilogram of ethanol-sulfanilamide in solution by mouth; (*C*) 0.1 gm. per kilogram of disulfanilamide given in capsule by mouth; (*D*) 0.1 gm. per kilogram of sulfanilamide given by mouth.

(9) reported one experiment on a normal man in which "prontosil soluble" was given intramuscularly without the appearance of any

[5] "Prontosil Soluble" is the sodium salt of 4′-sulfamidophenyl-2-azo-7-acetylamino-l-hydroxy-naphthalene-3:6-disulfonic acid.

color in the spinal fluid after five hours. We have performed a few experiments on the passage of ethanol-sulfanilamide, disulfanilamide, acetyl-sulfanilamide, and "prontosil soluble" into the cisternal fluid of dogs. Typical results are shown in figure 5.

"Prontosil Soluble" was given in solution by mouth to two dogs in a dosage of 0.33 gm. per kgm. (molecular equivalent of 0.1 gm. per kgm. of sulfanilamide). In the first, blood plasma and cisternal fluid collected at 3, 6, and 24 hours showed no pink color and did not contain over 1 mgm. per cent of sulfanilamide.[6] In the second, plasma taken at 3 and 6 hours contained only faint traces of the red dye and no detectable amount of sulfanilamide, while cisternal fluid taken at the same time showed neither color nor sulfanilamide. Urine collected up to six hours contained only 3 per cent of the amount of dye ingested, while considerably more than 50 per cent of the "prontosil soluble" was found unabsorbed in the gastro-intestinal tract. To another dog, the same dose of "prontosil soluble" was given subcutaneously. Within one hour a large amount of the red dye was present in the plasma and at 6 hours the plasma was still red. The whole animal was colored a bright red. Cisternal fluids taken at 3 and 6 hours showed only a trace of pink color, about one-fiftieth as much as that present in plasma. Samples of blood and spinal fluid taken at 3, 6, and 24 hours contained less than 1 mgm. per cent of sulfanilamide.

Ethanol-sulfanilamide appears to pass into the cisternal fluid as readily as sulfanilamide. Acetyl-sulfanilamide is definitely slower in its passage into the spinal fluid and in 6 hours is present in much lower concentration compared with that in the blood than is sulfanilamide. Disulfanilamide appears in the spinal fluid only in traces. When "prontosil soluble" is given subcutaneously, only traces of the red dye or of sulfanilamide appear in the spinal fluid.

DISCUSSION

From the data given here, it is seen that ethanol-sulfanilamide, the most soluble of the three compounds, is absorbed most rapidly from the gastro-intestinal tract, while disulfanilamide, the least

[6] Sulfanilamide was determined in the presence of the red dye by making two determinations with a photoelectric colorimeter, one for sulfanilamide in the usual way, and a second, using water instead of nitrite to correct for the red color of the dye.

soluble, is absorbed very poorly when large doses are given. It is obvious that determinations of the toxicity or lack of toxicity of insoluble or difficultly soluble compounds by the oral route may be misleading and dependent entirely on lack of absorption when large doses are given. This has been pointed out recently (5) in connection with the toxicity of acetyl-sulfanilamide where it was shown that an occasional dog may absorb large doses and die. It is dangerous to base lack of toxicity on lack of absorption. "Prontosil Soluble," an azo dye and soluble to the extent of 4 per cent in both acid and salt forms, is apparently absorbed very poorly from the gastro-intestinal tract. Although experiments with a large number of compounds are necessary before any generalization can be made, it is certain that in many cases the lack of toxicity of many insoluble compounds is due to poor absorption when given by mouth. The very rapid excretion of ethanol-sulfanilamide combined with its very rapid absorption prevents the attainment of constant blood concentrations over any length of time. With such a substance, doses would have to be given at frequent intervals to maintain any desired level in the blood. Too rapid absorption or too rapid excretion would appear to be undesirable properties for a compound for use in place of sulfanilamide.

The extremely rapid absorption of sulfanilamide when given in aqueous solution appears to be due to the rapid passage of the solution from stomach to intestine, where the absorption of the drug in solution has been proved to be very rapid. Some of the variability of absorption seen in different species or in individuals of the same species probably depends partly on differences in emptying time of the stomach. It is interesting to note that absorption is much more rapid when a solution of sulfanilamide is given by mouth than when such a solution is given subcutaneously (2).

The few experiments on the passage of these compounds into the spinal fluid indicate clearly that some of the sulfanilamide substitutes go into the spinal fluid only in traces (disulfanilamide, "prontosil soluble"); others are present in appreciable quantity but in relatively less amount than sulfanilamide (acetyl-sulfanilamide), while ethanol-sulfanilamide appears to resemble sulfanilamide in its rapid passage into the spinal fluid in high concentration.

The relatively low blood concentration of "prontosil soluble" when this substance is given by mouth as compared to subcutaneous injection is interesting in view of reports that in mice this drug is more effective by mouth than by injection (10, 11). Of course, all of the results reported here apply to the dog, and may not be applicable to other species.

SUMMARY

Ethanol-sulfanilamide, very soluble, sulfanilamide, moderately soluble, and disulfanilamide, very slightly soluble in water, have been compared in the dog as regards their absorption and excretion. Ethanol-sulfanilamide is absorbed and also excreted very rapidly, while disulfanilamide is absorbed slowly and excreted rapidly in comparison with sulfanilamide. Very little increased absorption occurs with a large dose of very insoluble compound as compared with a small one.

Sulfanilamide when given in solution by mouth is absorbed much more quickly than when given in solid form. Absorption is very slight from the stomach but very rapid from the intestine.

Sulfanilamide derivatives do not all pass into the spinal fluid as readily as does sulfanilamide.

We wish to thank Miss Dorothea Babbitt and Miss Alma Hendrickson for technical assistance.

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ABSORPTION AND EXCRETION OF SULFANILAMIDE IN THE MOUSE AND RAT[1]

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Since the mouse has been used almost exclusively for experimental therapeutic studies on sulfanilamide and allied compounds, it is important to study the absorption and excretion of sulfanilamide in this animal. By the use of a photoelectric colorimeter, it was possible to adapt our method for determining sulfanilamide to blood samples of 20 cmm. (1). Six to eight samples of blood could be taken in the course of a few hours from a mouse without any apparent disturbance of the animal. This procedure is obviously superior to the one used by us (2) and recently by Feinstone, Bliss, Ott and Long (3), of killing one or more mice to determine a point on the blood concentration curve. Blood concentration curves were obtained after administration of the same dose by mouth in aqueous solution, by mouth in acacia suspension, subcutaneously in aqueous solution, and subcutaneously in olive-oil suspension. The effect of varying doses was also studied. The urinary excretion and intestinal absorption were studied directly. A similar series of experiments was carried out on rats.

Blood was drawn by clipping the tail and using a 20 cmm. wash-out pipette moistened with a solution of heparin. The sample was discharged into 3.0 cc. of water in a centrifuge tube, allowed to hemolyze and was precipitated with 1 cc. of a 12 per cent solution of trichloracetic acid. After a few minutes' standing, it was centrifuged, and filtered through a small filter paper. Three cc. of the water-clear filtrate

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[2] Dr. Cutting is Fellow in Experimental Therapeutics and Medicine.

were treated with 0.3 cc. of 0.1 per cent sodium nitrite and after 3 minutes with 0.3 cc. of sulfamate-buffer solution (4). After 2 minutes, 1.5 cc. of the alcoholic solution of dimethyl-α-naphthylamine were added. After 10 minutes, the solution was read in a special photoelectric colorimeter.[3] Samples of blood from normal mice or rats gave no greater blanks than those given by water and the reagents.[4]

TABLE 1

Recovery of sulfanilamide from blood

SPECIES	MGM. PER CENT	
	Added	Found
Mouse	7.5	8.2
	7.5	8.0
	7.7	7.6
	7.7	7.8
	7.7	7.7
	14.4	14.5
	14.4	14.4
	14.4	14.2
	15.0	15.5
	15.0	15.6
	30.0	30.8
	30.0	30.1
	60.0	62.0
	60.0	61.4
Rat	7.5	8.0
	15.0	15.6
	15.0	15.6
	30.0	30.8
	30.0	31.0
	60.0	62.2
	60.0	62.0

The following table shows the recovery of sulfanilamide by this method where 0.02 cc. portions of mouse or rat blood were added to

[3] This colorimeter was designed and built by Dr. Morris Rosenfeld of this department. A description of the instrument will be published shortly.

[4] In some of our early experiments in which a toluenesulfonic acid filtrate of blood was used and nitrite was not destroyed, a correction of 1 mgm. per cent had to be subtracted from the value obtained.

3 cc. of standard solutions. The figures are calculated on the basis of sulfanilamide content of the blood.

Sulfanilamide was added to one sample of human blood to give 4.6 mgm. per cent; six determinations on 20 cmm. samples gave 5.0, 4.8, 4.8, 5.1, 5.1, 5.2 mgm. per cent; to another sample, sulfanilamide was added to give 17.3 mgm. per cent and six determinations gave 17.3, 17.4, 17.3, 17.5, 18.0, 17.3 mgm. per cent.

The 10 per cent acacia suspension of sulfanilamide was analyzed by measuring 0.1 cc. into a volumetric flask with the same syringe used for administration, and diluting to volume. Different batches gave average values of 9.3, 9.9, 10.0, 9.0, 10.0 per cent of sulfanilamide. The values in 20 measurements on different batches ranged from 8.4 to 10.4 mgm. per 0.1 cc. The suspension in olive oil used for subcutaneous injection averaged 9.1 per cent of sulfanilamide. No correction was made for the acacia suspensions not containing exactly 10 per cent of sulfanilamide. The above suggest that when suspensions are used, dosage in different mice may vary $\pm$ 10 per cent.

Blood concentration curves were determined on mice after administering the same dose of sulfanilamide in different vehicles and by different routes. Figure 1 gives the data of four experiments on the administration of the drug in solution by mouth, while figure 2 shows that similar curves are obtained on infected mice. Figures 3 and 4 are average curves of experiments on different methods of administering the drugs. The difference in the blood concentration curves when the drug is given by the different methods is not striking.

Different doses of sulfanilamide were administered to both mice and rats and blood concentration curves obtained. Figures 5 and 6 give the average values of all of these experiments. Although an occasional curve showed considerable variation from the others, the general spread of the curves was similar to that shown in figures 1 and 2.

The urinary excretion of sulfanilamide was studied in mice and rats as follows. After giving 0.4 gm. per kgm. of the drug in 1 per cent solution by mouth, the animal was placed in a funnel and arrangements made to collect urine. At the end of 8 hours, the animal was sacrificed, and the bladder and funnel carefully washed with a large volume of warm water. Urine and washing were combined, diluted

to known volume, and analyzed for free and total sulfanilamide. In nine experiments on the mouse the average eight hours excretion was for the free 46.5 per cent (variation 42–55) and for the total 63.0 per

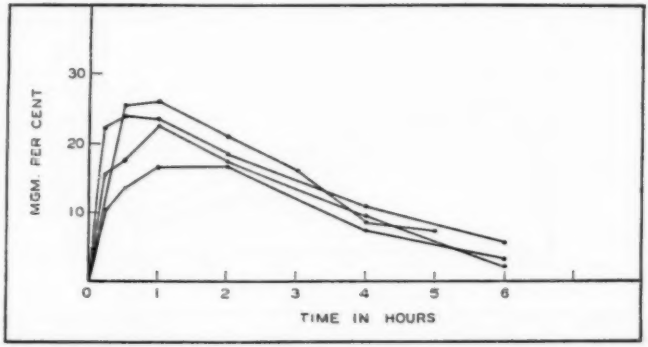

FIG. 1. Blood concentration curves for four mice after administration of 0.4 gm. per kilogram of sulfanilamide in 1 per cent aqueous solution by mouth.

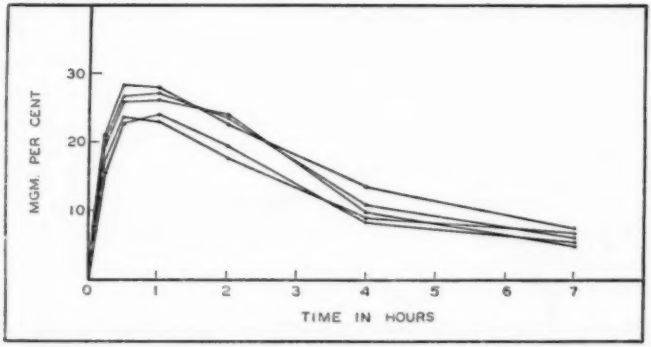

FIG. 2. Blood concentration curves for five mice obtained in exactly the same way as those in figure 1 except that mice were injected with 100 to 1000 lethal doses of β-hemolytic streptococcus (C 203) six hours before administration of sulfanilamide.

cent (variation 54–71) of the amount administered. In nine experiments on the rat, the average excretion for the free was 36.4 per cent (variation 26–45) and for the total 48.6 per cent (variation 32–57.5).

To check the speed of absorption from the intestine, mice and rats were given 0.4 gm. per kgm. of sulfanilamide in solution by mouth,

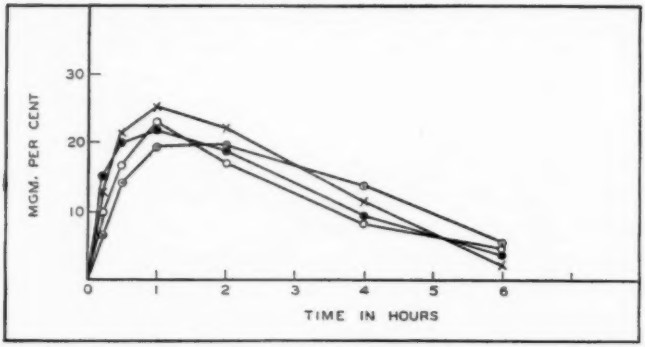

FIG. 3. Average blood concentration curves after administration of 0.4 gm. per kilogram of sulfanilamide by mouth in solution ((—●——●—), four mice; by mouth in acacia suspension (—○——○—), 4 mice; by subcutaneous injection in aqueous solution (—×——×—), 4 mice; by subcutaneous injection in olive oil suspension (—⊙——⊙—), 6 mice.

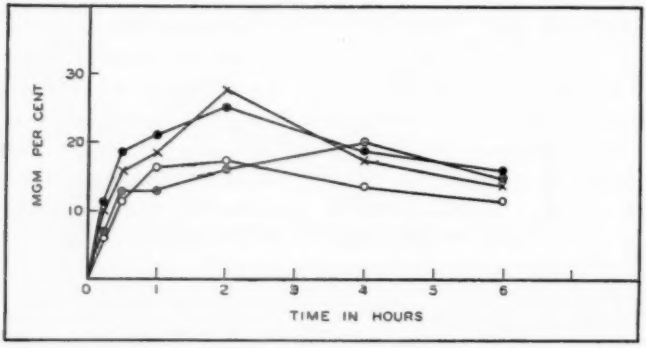

FIG. 4. Average blood concentration curves of rats after administration of 0.4 gm. per kilogram of sulfanilamide by mouth in solution (—●——●—), 3 rats; by mouth in acacia suspension (—○——○—), 6 rats; by subcutaneous injection in aqueous solution (—×——×—), 3 rats; by subcutaneous injection in olive oil suspension (—⊙——⊙—), 3 rats.

sacrificed at 1 hour, and the gastrointestinal tract thoroughly washed out. Four experiments on mice gave an average of 18.3 per cent

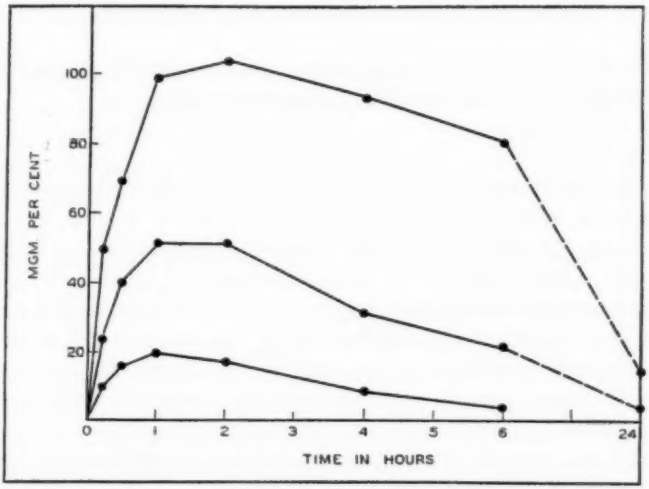

FIG. 5. Average blood concentration curves of mice after administering different doses of sulfanilamide in acacia suspension by mouth. Lower curve 0.4 gm. per kilogram, 4 mice; middle curve 1.0 gm. per kilogram, 4 mice; upper curve 3.0 gms. per kilogram, 4 mice.

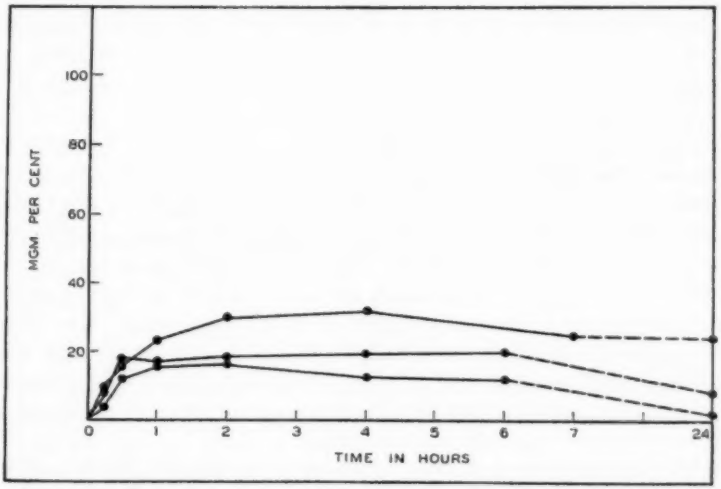

FIG. 6. Blood concentration curves of rats after administering different doses of sulfanilamide in acacia suspension by mouth. Lower curve 0.4 gm. per kilogram, 3 rats middle curve 1.0 gm. per kilogram, 3 rats; upper curve 3.0 gms. per kilogram, 3 rats.

(7.5 to 31.0) unabsorbed drug, while three experiments on rats gave an average of 35.3 (31.0 to 39.5) unabsorbed drug.

DISCUSSION

In the blood concentration curves, only free sulfanilamide has been considered, although both the mouse and the rat excrete the drug partly in the conjugated form. Special determinations on larger samples of blood showed that in both mouse and rat very little conjugated compound is present in the blood in the first six hours; the amount of conjugated sulfanilamide in the blood is not sufficient to affect any conclusion drawn from blood concentration curves of the free drug. Due to variability in the curves obtained on individual mice, a larger number of animals than has been used in the present study would be necessary to establish the significance of small differences in the blood concentration curves.

The most significant feature of the blood concentration curve for sulfanilamide in the mouse is the very rapid rise to a maximum (between one-half to one hour) and a very rapid fall to a low level in the course of six to eight hours. A comparison of these curves with those obtained for dog and man (5, 6) indicates that in the mouse the twenty-four hour blood concentration curve of the dog and man is compressed into about six hours. The rapid rise and abrupt fall indicate rapid absorption and excretion in the mouse. However, the absorption in the mouse appears to be no more rapid than that in the dog when sulfanilamide is given in solution instead of in capsule or tablet form (6). But, in the mouse no difference is seen in the blood concentration curves whether the drug is given orally in suspension or in solution. It is questionable whether any significance can be attached to the slight differences in the curves when the drug is given subcutaneously in aqueous solution or in oil suspension. Our data in regard to subcutaneous injection are not in agreement with the findings of Feinstone, Bliss, Ott and Long (3) that the blood level of sulfanilamide one hour after administration of a saline solution[5]

[5] We have repeated on 20 mice the comparison of the administration per os and subcutaneously of sulfanilamide in 0.8 and 2 per cent solution in saline. The results show no significantly higher blood levels from subcutaneous as opposed to oral administration.

subcutaneously is twice as great as when administered *per os*. The excretion of sulfanilamide appears to be more rapid in the mouse than in dog or man as shown by the rapid fall of the blood concentration curve and by the fact that 63 per cent of the ingested dose is excreted in the urine in eight hours as compared with about 25 per cent in the dog (6).

The blood concentration curves obtained on the rat differ from those on the mouse mainly in a slower rise of the blood level, a slower rate of fall, and a much lower maximum concentration from large doses (1 and 3 gms. per kgm.). These curves indicate that both absorption and excretion are slower in the rat than in the mouse. This conclusion is supported by the data showing an excretion of 63.0 per cent of the ingested dose in the mouse in 8 hours as compared with 48.6 per cent in the rat, and by our finding of 18.3 per cent unabsorbed drug in one hour in the mouse as opposed to 35.3 per cent in the rat.

In order to maintain a constant blood level of sulfanilamide in the mouse, doses would have to be given at more frequent intervals than in the dog or man, while in the rat less frequent doses than in the mouse would suffice. No indication is given from these experiments that the mode or vehicle of administration of sulfanilamide in the mouse produces significant differences in the blood concentration curve. The finding that with large doses (1 and 3 gms. per kgm.) the blood level of the rat is much lower than with corresponding doses in the mouse is in accord with the recent report that the toxicity of sulfanilamide administered by mouth is much lower for the rat than for the mouse (7). In our own experiments, we noted that the mouse exhibited severe symptoms with a dose of 3 gms. per kgm. while the rat did not.

SUMMARY

Blood concentration curves were determined on mice and rats after administering sulfanilamide in different dosage, in different vehicles, and by different routes. The percentage excretion of the drug in eight hours by the kidney was determined, as well as the amount remaining unabsorbed in one hour in the intestinal tract. From the data obtained, it is concluded that excretion is more rapid

in the mouse than in the rat, dog, or man. No significant difference was found when sulfanilamide was administered by different routes or in different vehicles. When large doses of the drug are given, the maximum concentration in the blood of the rat is much lower than in the mouse.

We wish to thank Miss Dorothea Babbitt and Miss Alma Hendrickson for technical assistance with the analyses.

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THE PASSAGE OF SULFANILAMIDE THROUGH THE HUMAN PLACENTA

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WITH THE TECHNICAL ASSISTANCE OF DOROTHEA BABBITT

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The widespread use of sulfanilamide in the treatment of pyelitis and other intercurrent infections during pregnancy has prompted the present inquiry into its transmission through the human placenta.

A single dose of sulfanilamide was given by mouth to seventeen normal, full-term patients during the first stage of labor. The dosage employed was 0.06 gram per kilogram of body weight. In the two instances in which vomiting followed the administration of the drug the results were discarded. At the moment of delivery, which occurred after intervals which ranged from 1 hour to 38 hours following administration, a sample of maternal blood was taken by venipuncture. Fetal blood was obtained from the umbilical cord immediately following expulsion of the placenta. In two cases in which the membranes ruptured while the patient was on the delivery table, it was possible to obtain samples of the amniotic fluid. Determinations of free and total sulfanilamide were made according to the method of Marshall (1).

The results are summarized in Table I. It may be seen that the drug passes readily from mother to fetus, as was recently demonstrated in the rabbit by Lee, Anderson, and Chen (2). The sulfanilamide concentration of the fetal blood rapidly approaches that of the mother, equilibrium being reached within approximately five hours. This was discovered to be true for both the free and acetylated forms of the drug, a finding at variance with the results of the aforementioned authors (2) which showed an appreciably lower concentration of acetylated sulfanilamide in the fetal blood than in the maternal. In the two cases in which amniotic fluid was available for analysis, the

[1] Henry Strong Denison Scholar for 1937–38.

sulfanilamide concentration indicated a correspondingly free passage of the drug.

Clinically, the infants showed no ill effects from the drug either at birth or during the neonatal period. Pediatric follow-up studies during the following eight months revealed each baby to be entirely normal in its general development and weight increase.

Sulfanilamide has a molecular weight of 171, a figure which might suggest its ready diffusability through the placenta. However,

TABLE I

PATIENT	BODY WEIGHT	DOSE	INTERVAL BETWEEN ADMINISTRATION AND DELIVERY	SULFANILAMIDE					
				Maternal blood		Fetal blood		Amniotic Fluid	
				Free	Total	Free	Total	Free	Total
	kgm.	*grams*	*hours: minutes*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
M. Z.	60	3.6	1:00	2.7	3.0	1.8	1.8		
R. B.	63	3.9	2:00	4.3	4.5	3.3	3.5		
G. S.	60	3.6	2:20	1.4	1.6	1.0	1.2		
G. W.	55	3.3	2:45	4.5	4.7	3.3	3.3		
M. G.	62	3.6	3:30*	0.6	0.8			0.6	0.8
			4:50	0.8	0.9	0.5	0.5		
V. R.	73	4.5	5:15	6.0	6.8	6.1	6.7		
N. R.	60	3.6	5:15	2.8	3.8	2.8	3.5	2.2	2.6
E. K.	69	4.2	5:30	6.4	7.6	6.0	7.0		
D. W.	66	3.9	5:40	6.3	6.9	5.8	6.1		
L. M.	60	3.6	7:20	4.8	6.3	4.7	5.8		
A. A.	61	3.6	10:00	4.2	5.4	4.2	5.4		
E. W.	57	3.3	12:00	2.2	3.7	2.1	3.1		
F. C.	69	4.2	15:10	4.8	6.1	3.8	3.9		
E. W.	49	3.0	30:00	1.2	2.4	0.9	1.3		
A. H.	65	3.9	38:00	0.2	0.4	0.2	0.6		

* Interval between administration and rupture of membranes.

predictions of placental transmission based solely upon molecular weight are fraught with uncertainty; thus, Manahan and Eastman (3) have recently demonstrated the unpredictable rôle of placental selectivity by finding a threefold difference between the concentrations in fetal and maternal blood of cevitamic acid, a substance of low molecular weight.

It should be stressed that the data presented represent the placental

transmission of sulfanilamide in the human, only at term. That the stage of gestation is a factor of great importance in placental transmission has recently been demonstrated (4), and it is entirely possible that in earlier stages of pregnancy great differences from the present findings may occur.

SUMMARY

Sulfanilamide, given by mouth to pregnant, full-term women during labor, passes readily to the fetus, the concentrations of both the free and acetylated forms reaching equilibrium between the fetal and maternal bloods within approximately five hours. The drug also passes rapidly into the amniotic fluid.

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NOTE: Since this paper was prepared for publication, R. H. Barker has reported (*New England J. Med.*, 1938, **219**, 41) the results of analyses of maternal and fetal blood following the therapeutic administration of sulfanilamide to patients before delivery. His data are in complete agreement with the present findings.

DELAYED TOXIC REACTION TO SULPHANILAMIDE

Case Report

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Submitted for publication June 21, 1938

It is now well known that sulphanilamide commonly causes periods of confusion, disorientation and other behavior abnormalities. These and almost all the other toxic reactions reported occur during the period of therapy or within twenty-four hours after the last dose of sulphanilamide, and most of them disappear promptly when the drug is discontinued. Very few reports of toxic reactions beginning more than twenty-four hours after therapy is stopped have been found. The patient to be described exhibited a delayed reaction occurring on two occasions, each time about four and one quarter days after the last dose of sulphanilamide. The reaction was marked by cyanosis, confusion and negativism, and on each occasion recovery was prompt. No report of such a response to the drug was found while the patient was under observation.

Hageman and Blake (2) have reported two cases of dermatitis with fever which began more than twenty-four hours after the last dose of sulphanilamide. In one of their cases the reaction began on the second day, in the other on the fifth; two other patients reacted twenty-four hours after the last dose. Coleman and Purdie (1) mention two patients who had "mental disturbances of hysterical type . . . in one case fifteen days after the cessation of sulphanilamide" and in the other after an unspecified latent period. No further details are given. Hogan (3) observed a psychotic reaction marked by paranoid trends, apprehension, restlessness, and auditory and visual hallucinations. The illness began during a course of sulphanilamide therapy and had not entirely ended a month later when the case was reported.

CASE REPORT

J. N., a 19 year old seaman, was admitted to the Brady Clinic of the Johns Hopkins Hospital on January 25, 1938 with a gonococcal urethritis of four weeks'

duration. After treatment with sulphanilamide and protargol, he was discharged January 31 and was followed in the hospital dispensary until February 4 after which time he was lost sight of. The course in the hospital and dispensary was entirely uneventful.

On March 5, the patient was admitted to the urological service of the Baltimore City Hospitals because of a flare-up of his infection. From March 6 to April 3 he was treated with sulphanilamide. The daily totals of the doses, in grains, were: March 6, 80; March 7 to 11, 60; March 12 to 13, none; March 14 to 20, 30; March 21 to April 3, 40. Each grain of sulphanilamide was accompanied by a grain of sodium bicarbonate. The total dose of each drug was 1230 grains. The blood sulphanilamide and CO_2 combining power determinations were:

Date	*Sulphanilamide mg. per 100 cc.*	*CO_2 volumes per cent*
March 10		39
" 17	4.0	50
" 29	5.1	45
April 1	5.8	39

The last dose of sulphanilamide was given at 8 p.m. on April 3. The next morning the patient was discharged, improved. No signs or symptoms of intoxication were noted during the period of hospitalization.

In the early hours of the morning of April 8, the patient was arrested. A policeman found him standing on a corner in a dazed condition, unable to give an account of himself. In the police station the patient created a disturbance and was taken to the Johns Hopkins Hospital accident room where he was examined by a member of the Henry Phipps Psychiatric Clinic house staff. The patient stood at first with his hands clenched, his body held rigid, his head turned to the right. He refused to answer questions until the police had left the room. Then he said he had "thrown a fit" in the station house to force the police to take him to a hospital. He said, "I was just play acting. People are after me or else why am I in jail?" He was oriented, was not hallucinated, could give no details as to the people who were after him. He was able to say he had received sulphanilamide at the City Hospitals. Cyanosis of the lips and hands was noted.

The patient was returned to jail for the night. The next morning he was brought back to the hospital. Again he was negativistic, uncommunicative. He held himself rigid in bed and refused to allow a physical examination. He was then committed to the psychopathic division of the City Hospitals.

At the City Hospitals the patient behaved on admission as he had at the Johns Hopkins Hospital. He was slightly cyanotic, held himself rigid, resisted the attendants, would not permit an examination. This condition persisted until 7 p.m. on April 10 when the rigidity disappeared and the patient made an apparently sincere attempt to answer questions. He looked like a man who had just been aroused from sleep; he was bewildered and had lost track of time.

The next morning the rigidity had returned and with it the negativistic be-

havior. That evening the patient again responded to questions and attempted to cooperate, but he was still quite confused. The next morning, April 12, he was alert, in good rapport with his environment. The mental status examination revealed no abnormalities and the patient was able with some difficulty to recall his activities from the time of his discharge from the City Hospitals until his readmission.

Physical examination revealed only a gonococcal anterior urethritis and the cyanosis mentioned. The rectal temperature was 101 F. on admission and the pulse 136; both fell to normal the next day and remained normal until the patient was discharged. The laboratory findings were: RBC 4,500,000; Hb. 100%, 14.5 grams (Wintrobe); WBC 10,000; Eagle test, negative; blood sulphanilamide, trace. *Spinal fluid*, slightly cloudy, pressure 150 mm. of CSF, Pandy faintly positive, no WBC, no xanthochromia, 600 fresh RBC, dynamics normal, Wassermann 4–4–2, Mastic 2211100000. The spinal fluid was normal when it was examined again a month later; the Wassermann and Mastic tests were negative.

On April 15, sulphanilamide therapy was instituted again because of the continuing urethritis. The daily totals of the doses in grains were: April 15, 20; April 16, 80; April 17, 60; April 18, 40; April 19 to 20, 20; April 21 to 26, 40; April 27, none; April 28 to 30, 80; May 1, 60; May 2, none; May 3, 60; May 4 to 8, 120; May 9, 90; May 10, 120; May 11, 60. Difficulty in obtaining a constant supply of the drug accounted for the irregularity of the dosage. After April 17, each grain of sulphanilamide was accompanied by a grain of sodium bicarbonate. The total dose of sulphanilamide was 1710 grains, of sodium bicarbonate 1550 grains.

The following changes in the blood occurred:

Date	*Sulphanilamide mg. per 100 cc.*	*CO_2 volumes per cent*
April 18	5.0	32
" 21	3.2	45
" 26	3.7	
" 30	7.1	
May 9	9.0	
" 11	11.0	

The last dose was given at noon on May 11, the last sulphanilamide determination was about four hours later to allow for complete absorption. No further sulphanilamide was given. There were no signs or symptoms of intoxication during this period. Therapy was stopped suddenly as it had been on the previous admission.

The patient remained quite well until the evening of May 15, four and one quarter days after receiving the last dose. That evening he appeared to be a little surly. The next morning he remained in bed, tossing restlessly and complaining of headache and lower abdominal pain. He was quite confused and could answer questions only with much hesitation. There was no rigidity. Physical examination was negative except for slight cyanosis of the lips and distension of the bladder. That night the patient was catheterized and 950 cc. of normal urine were removed.

No urethral obstruction was found. Since he did not void during the next twenty-four hours, a retention catheter was inserted. It was removed May 17 when the patient began to void voluntarily.

Until May 25 the confusion continued with slight variations in intensity. The restlessness disappeared on May 17, and from then until the end of the reaction the patient was drowsy and listless most of the time and answered questions with difficulty. May 22 he was visited by his brother and that night he seemed mildly elated; he sang and talked a great deal but the confusion continued. The next morning he seemed to have recovered; he said "I feel myself again," he asked for his clothes and he talked to other patients. However at noon he was again confused and listless; he sat staring and finally pulled the bed clothes over his head. The next day he was confused and drowsy. On May 25 the reaction ended. The patient dressed, regained his rapport with the environment and said "I feel better. I am going to get well." From then until discharge on May 31 his behavior was normal.

The laboratory findings during the second reaction were:

Date	*Sulphanilmide mg. per 100 cc.*	*CO_2 vols. per cent*	*NPN mg. per 100 cc.*	*RBC*	*Hb (Wintrobe) gms.*	*WBC*
May 16.....	Less than 1					
" 17.....	Less than 1	51	25	4,200,000	12.8	11,400
" 24.....	Less than 1		27			

Neither methemoglobin nor sulphemoglobin was present in the blood on May 17.

DISCUSSION

The mechanism of the reaction described is unknown. It apparently is not related to anemia, leukopenia, reduced alkali reserve, methemoglobinemia, sulphemoglobinemia, nitrogen retention or sulphanilamide retention. Some decomposition product of sulphanilamide, as suggested by Marshall and Walzl (4), may be a factor but the evidence for this is not complete, nor is it clear why this product should have its effect only after therapy is discontinued. Speculation on analogies between this reaction and the delayed toxic effects exemplified by delirium tremens or by withdrawal symptoms in morphine addiction are interesting but not otherwise profitable in the present state of our knowledge.

Further studies are needed to determine whether delayed toxic reactions can be prevented by gradually reducing the doses of sulphanilamide at the end of the course of therapy.

SUMMARY

A toxic reaction to sulphanilamide, occurring twice in the same patient and each time after a latent period of more than four days, is described. The reaction is marked by cyanosis, confusion and negativism.

I am indebted to Dr. Esther Loring Richards for permission to report this case.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Essentials of Obstetrical and Gynecological Pathology. By MARION DOUGLASS and ROBERT L. FAULKNER. 187 pp. Illus. $4.75. (*C. V. Mosby Company, St. Louis, 1938.*)

There has long been a genuine need for a text covering the large and growing field of gynecological and obstetrical pathology. This need is accentuated by the fact that in textbooks of gynecology and obstetrics the pathology of diseases of the generative organs is commonly given rather perfunctory treatment. Aside from the no longer up-to-date monograph of Frank, with its inadequate illustrations and its rather difficult readability, and the recent British work by Teacher and Marshall, which leaves much to be desired and which contains not a few inaccuracies, there has been no book in English available for students in the courses in gynecological and obstetrical pathology which are now given in most medical schools.

The manual of Douglass and Faulkner is not intended as a work of reference, for it aims to present only the elements of the subject. Nevertheless, and perhaps for this reason, it should answer the needs of the medical student very satisfactorily. The only question is as to whether the effort at brevity may not have been carried too far. For example, ovarian cancer receives no discussion at all except for brief mention of a few special forms, and only a page and a half is devoted to the discussion of adenocarcinoma of the uterus, in which differential microscopic examination is so important. The many illustrations, chiefly photomicrographs, are in the main quite adequate. The book should be genuinely helpful in its field. There is still room, however, for a work of somewhat broader scope, without necessarily emulating the exhaustive treatment found in the German handbooks.

E. N.

The Chemistry of the Sterids. By HARRY SOBOTKA. 634 pp. $8.50. *Williams & Wilkins Company, Baltimore, 1938.*

The six years which have elapsed since Rosenheim and King proposed the cyclopentanoperhydrophenanthrene structure for cholesterol and cholic acid have seen a great revival of interest in this field. Such physiologically important compounds as the male and female sex hormones, the cardiac aglycones, Vitamin D, the toad poisons and more recently compounds with adrenal cortical hormone activity have been shown to possess the same basic carbon skeleton.

In "The Chemistry of the Sterids" the author has given us a work which should be valuable both to the general chemist and to the specialist. The first section of the book deals with such topics as the history of sterid chemistry, methods of structural research, sterochemistry, the physical properties of sterids, analytical methods and a comprehensive discussion of that very interesting group of compounds, the choleic acids. The last two-thirds of the work are devoted to a tabulation of the sterids described in the literature available up to January 1, 1937. The compounds are classified according to their functional groups and physical and chemical properties. References to the literature are given for each compound. It is to be hoped that the author will issue supplementary tables including the sterids described in the more recent literature.

L. L. E.

Fever Therapy. Abstracts and Discussions of Papers presented at the First International Conference on Fever Therapy, New York, March, 1937. Edited by Walter M. Simpson, William Bierman, et al. 486 pp. $5.00. Paul B. Hoeber, Inc., New York, 1937.

Originally, it was the hope of the American Committee to publish all the contributions in complete form. The program, however, was so much larger than anticipated that it became necessary to limit the volume to abstracts in English, in French and in German, with the discussions of papers only in English.

In the English section there are 184 pages containing 70 abstracts and collections of discussions. The book, of course, affords the usual advantages and disadvantages of publications in abstract form. The abstracts seem sufficiently complete to give the reader the ideas that the authors wish to convey.

The articles are grouped into four sections, namely, Physiology and Pathology, Fever Therapy of Miscellaneous Diseases (of which there are many), Fever Therapy of Syphilis, and Fever Therapy of Gonococcic Infections. The book will give the reader a good general idea of the status of fever therapy at present. The print is excellent.

T. P. S.

The Life of Chevalier Jackson. An Autobiography. 229 pp. Illus. $3.50. The Macmillan Company, New York, 1938.

The great contributions of Chevalier Jackson to the art of healing are too well known to be recalled here. But, it is only after reading his autobiography that one can appreciate fully the magnitude of his achievements. Poverty, a frail physique, a grave illness and a marked sensitivity are some of the obstacles which he has had to overcome. From the picture that he draws of his early struggles the reader is moved to admire the extraordinary will power as well as the high intellect and the mechanical and artistic gifts he possesses. The last quality is manifest in the skill with which he has written these reminiscences, in particular

the part concerning his childhood and youth. The account of his professional activities, however, is not given in so much detail as one would wish. The development of his technique and of the specialty which he dominates could and should have been amplified. Instead, the author prefers to discuss at some length in the latter part of the book his method of training assistants and to emphasize the need and means of prevention of the type of accidents which he treats so well.

Altogether, this is a remarkable document and the pleasure one obtains in reading it is further increased by the inclusion of numerous photographs of paintings by the author. In view of this, one can overlook his biassed remarks on the use of alcohol and tobacco which he apparently abhors with the strength and convictions of a professional reformer.

L. M. P.

BOOKS RECEIVED FOR REVIEW

Essentials of Obstetrical and Gynecological Pathology. By MARION DOUGLASS and ROBERT L. FAULKNER. 187 pp. Illus. $4.75. C. V. Mosby Company, St. Louis, 1938.

The Pharmacological Shock Treatment of Schizophrenia. By MANFRED SAKEL. With a foreword by Professor Otto Pötzl. Authorized translation by Joseph Wortis. Revised English edition. 136 pp. Illus. $2.75. Nervous and Mental Disease Publishing Co., New York and Washington, 1938. (Nervous and Mental Disease Monograph Series No. 62.)

Major Endocrine Disorders. By S. LEVY SIMPSON. 184 pp. Illus. 10 s. 6 d. John Bale Medical Publications, Ltd., London, 1938.

Die Therapie der Thrombose. By ERNST FRIEDLÄNDER. 117 pp. Paper, 5.40 marks. Franz Deuticke, Leipzig und Wien, 1938.

Paracelsus en Hahnemann, een Renaissance der Geneeskunst. By R. A. B. OOSTERHUIS. 300 pp. A. W. Sijthoff's Uitgeversmaatschappij N. V., Leiden, 1937.

Methods of Tissue Culture. By RAYMOND C. PARKER. 292 pp. Illus. $5.00. Paul B. Hoeber, New York, 1938.

Medical State Board Questions and Answers. By R. MAX GOEPP. 7th ed. revised. 644 pp. $5.50. W. B. Saunders Company, Philadelphia, 1938.

Injection Treatment of Varicose Veins and Hemorrhoids. By H. O. MCPHEETERS and JAMES KERR ANDERSON. 315 pp. Illus. $4.50. F. A. Davis Company, Philadelphia, 1938.

The Chemistry of the Sterids. By HARRY SOBOTKA. 634 pp. Diagrams. $8.50. Williams & Wilkins Company, Baltimore, 1938.

Materia Medica, Drug Administration and Prescription Writing. By OSCAR W. BETHEA. 5th revised ed. 577 pp. $5.00. F. A. Davis Company, Philadelphia, 1938.

The Life of Chevalier Jackson. An autobiography. 229 pp. Illus. $3.50. The Macmillan Company, New York, 1938.

The Culture of Organs. By ALEXIS CARREL and CHARLES A. LINDBERGH. 221 pp. Illus. $4.50. Paul B. Hoeber, Inc., New York, 1938.

A Synopsis of the Diagnosis of the Acute Surgical Diseases of the Abdomen. By JOHN A. HARDY. 345 pp. Illus. $4.50. C. V. Mosby Company, St. Louis, 1938.

A General Textbook of Nursing. By EVELYN C. PEARCE. 888 pp. Illus. E. P. Dutton & Co., New York, 1938.

The Prevention of Puerperal Sepsis. By L. COLEBROOK. And *Antisepsis in Midwifery.* By L. COLEBROOK and W. R. MAXTED. 109 pp. Paper, $1.00. Oxford University Press, New York & London, 1938. (Oxford Medical Publications.)

A Survey of Chronic Rheumatic Diseases. Contributed by contemporary authorities in commemoration of the bicentenary of the Royal National Hospital for Rheumatic Diseases, Bath, 1738–1938. 338 pp. Illus. $6.50. Oxford University Press, New York & London, 1938. (Oxford Medical Publications.)

A Textbook of Bacteriology. By THURMAN B. RICE. 2nd ed. revised. 563 pp. Illus. $5.00. W. B. Saunders Company, Philadelphia, 1938.

UNIV. OF MICH.

Dr. Thomas Richmond Boggs

Minute adopted by The Medical Board of The Johns Hopkins Hospital, October 3, 1938

It is with genuine sorrow that we record the death on September 2nd of Doctor Thomas Richmond Boggs, devoted friend, esteemed colleague, loyal member of the University Faculty and of the Visiting Staff of the Hospital.

Born in Memphis, Tennessee, on October 2nd, 1875, Doctor Boggs was raised in a cultured atmosphere, studied at the Universities of Georgia and Pennsylvania, and received his degree of Doctor of Medicine from The Johns Hopkins University in 1901.

After his graduation, he served as a Medical House Officer, studied in Tubingen, practiced for a short time in Florida, and in 1904 he returned to Baltimore to renew his affiliation with The Johns Hopkins Hospital, where he became Resident in Medicine in 1908 and served until 1911. Like others privileged to develop under Doctor Osler's unique influence, he acquired not only medical learning but was stimulated to practice those principles and ideals that make for the best of professional relationships.

He was a skillful, thorough physician whose attitude towards his patients of every class alike was one of warm sympathetic understanding: a stimulating teacher whose quality of mind, lucidity of expression and ability tellingly to present a subject made what he taught difficult to forget. If a teacher's quality can be measured by his generosity to his colleagues and to his students, his was of the best.

As a friend, he was loyalty personified. He had a broad culture—was a capable botanist, an outstanding clinical microscopist, was versed in the Bible and in general literature. With reason, those who were his junior associates recall the wide scope of his learning, for in good spirit and with a merry twinkle he often dropped them a jewel that sent them asearch to learn where it had been mined.

Doctor Boggs was modest and retiring. He volunteered counsel seldom but gave freely of his best when called upon. His honesty could not be swayed by opportunism or by opposition. Of strong opinion, he was not rigid and he was of the very few whose judgment of men was uninfluenced by bias or by prejudice. These attributes and his ability earned for him the post of Associate Professor of Medicine in The Johns Hopkins Medical School in 1911 and of Visiting Physician of The Johns Hopkins Hospital in 1928.

In 1911, he was appointed Physician-in-Chief of the Baltimore City Hospitals and in that post he developed a clinic that gave such a high order of medical care to the indigent sick of Baltimore and of instruction to students and Staff that it stands as a monument to him.

During the Great War of 1914, he attained the rank of Colonel, was Chief Medical Officer of Base Hospital 18 and Chief Medical Consultant of the Air Service of the American Expeditionary Forces in France. After his retirement from the Army he served as Consultant to the United States Veterans' Bureau in Washington.

Though he wrote little, he stimulated others to write, and he was a member of many Medical Societies. After he had served for eleven years as Recorder, he was elected President of the Association of American Physicians in 1937.

In 1928, he married Miss Kate Newell Doggett, of Fredericksburg, Virginia.

Tom Boggs was a gentleman with a charm and a courtliness reminiscent of the best Southern tradition, a physician and a teacher of eminence. He did justice, loved mercy and walked humbly with his God. With his passing, the Medical School, the institutions with which he was affiliated, the community and his many friends suffer a real loss.

THE USE OF GONADOTROPIC HORMONES IN THE ADULT RHESUS MONKEY

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Submitted for publication October 1, 1938

Heape (1894) and van Herwerden (1905, 1906) first reported the occurrence of non-ovulatory menstrual cycles in macaques shot in the wild and Corner (1923) demonstrated the condition in rhesus monkeys in captivity. Since that time a large amount of data has accumulated in the records of the Carnegie colony of macaques on the high incidence of non-ovulatory cycles, a brief summary of which the writer published in 1937. In that study a distinction was drawn between "normal" and "pathological" failure to ovulate. It was pointed out that even healthy adult rhesus females almost never ovulate in the non-breeding season, May to September. Adolescent females, and those approaching the menopause, likewise females resuming periodic bleeding after pregnancy or lactation tend "normally" to bleed without ovulating.

If, on the other hand, an otherwise healthy female fails to ovulate in each and every cycle during the more favorable months of the year (October to March), such behavior would be considered pathological since the majority of the more vigorous individuals ovulate with considerable regularity, as noted also by Morse and van Wagenen (1937).

Such is the rule or norm. The Carnegie colony has, however, always harbored a number of animals that remained refractory for variable periods of time. For example, one otherwise quite superior female (No. 217) menstruated with unusual regularity in 26- to 28-day cycles over a period of four years, yet never once ovulated, a fact ascertained by frequent palpation and by a dozen laparotomies. Regularly ovulating females may, moreover, without apparent cause, interpose a non-ovulatory cycle in the midst of normal ones. With

newly acquired, mature females that have only recently borne the hardships of a journey from India it is the exceptional thirty percent in which one may expect ovulation within the first few months after arrival.

Until the last several years non-ovulatory cycles were thought to be a peculiarity of the monkey, but for women Robert Myer's dictum "Ohne Ovulation, keine Menstruation" prevailed. Human cases are, however, now being reported in numbers (Novak, 1938), and one type of sterility, in otherwise normal women, is being ascribed to persistent failure of ovulation. The writer has long been interested in this clinical problem, since it has loomed large in the Carnegie rhesus colony. The present study was, however, more directly prompted by the report of Davis and Koff (1938) who were able in numerous human cases to determine by laparotomy a day or two after administration of a gonadotropic hormone that ovulation had taken place.

The salient data of the 104 experiments of the present series are in large part included in Tables 1 to 4, the key to which is found in the legend under Table 1. The kind of extract used, as well as dosage and mode of injection, is indicated.[1] The diagnosis of results was

[1]Gonadotropic material from 4 sources was used. The majority of the experiments were made with *Gonadogen*, potent extract of pregnant mare's serum supplied by the Upjohn Company. Dr. F. L. Hisaw furnished some follicle stimulating hormone (F.S.H.) made by Dr. Fevold out of whole sheep pituitary. From E. R. Squibb and Sons were secured the two reagents *Gamone*, made from the urine of menopausal women, and *Follutein*, made from human urine of pregnancy. *Follutein* is richer in the luteinizing principle (L.H.) and does not by itself cause growth of follicles in man or monkey. Thanks are due the generous donors for liberal supplies of the extracts, which were just what they purported to be. If our experiments proved to be largely failures this is due to no fault of the reagents employed but to the difficult subjects of the experiments, or in other words to the writer's inability to guage the dosage required in individual cases.

An innovation in the method of administering the water-soluble gonadotropic hormone *in oil* is here reported. The dry extract is shaken up with the oil by the supersonic machine of Prof. Wood which disperses the hormone particles thoroughly throughout the oil, thus favoring slow absorption. The writer owes the suggestion to the success which attended the efforts of Dr. E. L. Keeney in the preparation of adrenalin for prolonged action (*Bull. Johns Hopkins Hospital*, 62: 227, 1938).

made in part by the author's method of bimanual palpation, frequently checked by laparotomy, at which time the ovaries and uterus could be inspected. Frequently an ovary was removed and the uterus biopsied, with or without recovery of an ovum; or the animal was killed and the organs studied histologically, as indicated in the tables or stated in the more detailed protocols printed in small type in the different sections.

I. ANIMALS SHOWING LITTLE OR NO RESPONSE TO THE HORMONES

About one-third of the injections (32 out of 104) elicited little or no response in the ovaries, although the dosages used were comparable to those effective in other animals or indeed in the same animal at other times, as may be seen from the records of a dozen animals used more than once (Tables 1–4). Some of the subjects were clearly unfavorable, being sick, poorly acclimated, or approaching the menopause. Yet the reason for half of the complete failures was not apparent.

1. Sick animals. Eight animals were perceptably below par, though all were bleeding periodically. No. 522 menstruated but without palpable changes in the size of the ovaries, either spontaneously or in response to injections. No. 535 had a skin infection but seemed otherwise healthy. Nos. 267 and 424, once excellent animals, became refractory to *Gonadogen* while in the later stages of tuberculosis.

2. Poorly Acclimated Females. Nine cases of failure are recorded (Nos. 273, 437, 439, 441, 443, 452, 454, 559, 563). As may be seen in Table 1 huge doses of Hisaw's *F.S.H.*, of *Gamone* and of *Gonadogen* were ineffective in these animals. It should be stated, however, that some poorly acclimated individuals did respond although none were made to ovulate.

3. Old Females. Nos. 57 and 114 were over 11 years old when used in these experiments but whether they were approaching the menopause is uncertain. Our experience with two females (Nos. 5 and 7) leads us to believe that at about 18 or 19 years of age the rhesus monkey approaches the menopause.

4. Ovulating Females Becoming Refractory. To this group belong 13 females whose reproductive functions proceeded normally for a

period, only to lapse again, for unknown causes, to something below perfection. As the tables show eight of these (Nos. 237, 265, 305, 330, 395, 430, 442, 451) responded to hormones at one time, but not at all on the occasions recorded in this section.

TABLE 1

Cases in which the injections had little or no effect

ANIMAL			DAYS 5	10	15	20	25	30
57	I	- -	? day 34	3 6 6 6 5 c.c.	F.S.H. Hisaw	M	March	
57	II	- -	4/5 6 6 6 6 6 5 6 c.c.	F.S.H. Hisaw				
114	I	- -	3/28 4 2 4 4 4 4 4 4 5 4 2 c.c. F.S.H. Hisaw			22 M		
114	II	- -	3/10 6 9 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 6	R.U.	F.S.H. Hisaw		28 M	
237	I	- -	10/14 30 0 L				25 M	
237	II	Amenorrheic	d 54→6 6 6 6 6 6 12 0 12 10 50			†	May	
265	I	+ -	3/10 30 0♣ ♀ 19 M					
265	II	- ?	3/23 10 10 10 30			21 M		
265	III	- -	? 6 6 12 0 6 6 12 12 12 30 †e-Colchicin No mitosis				Feb.	
287	I	——	1/29 2.5 2.5 2.5 2.5 2.5 5 15 Followed by Amenorrhea					
287	II		50				March	
273		- -	3/11 10 20 20 20 20 20 40 40 40 40 40 40 40 40 40 18 M		F.S.H. Hisaw			
291		- -	3/11 10 20 20 20 20 20 20 L 20 M		Slight effect			
308		- -	9/12 24 24 24 24 24 F.S.H. Hisaw L †					
400		+ -	3/28 40					†
430	I	- -	3/23 5 5 5 5 5 15 ▲ △ ♀			22 M	Large follicle	
430	II	- -	3/17 5 5 5 5 5 15 · No effect				→	
430	III	- -	10/7 1 1 1 1 1 1 1 1 1 1 1 1 1 Ovulation				25 M	
430	IV		1/17 12 12 24 0 12 12 12 12 12 30 †					
433	I	+ -	3/11 30 L 18 M					
437	I	- -	11/11 20 40 40 40 40 40 40 40 40 40 20 Gamone 20 M					
437	II	- ±	3/20 15			21 M		
439		- -	11/7 20 40 40 40 40 40 40 40 Gamone 18 M					
441		- -	3/8 30			21		
443		- -	3/25 5 10 10 † Died					

The Roman numerals placed above the animal number in the first column indicate two or more experiments done on the same animal. The plus or minus signs stand for ovulatory (+) and non-ovulatory (−) cycles immediately preceding the last menstrual period, the first day of which, when known, is given in the next column. Figures representing daily administration in rat units (R.U.) are inserted in columns corresponding to days of the menstrual cycle. Unless otherwise stated the reagent used was *Gonadogen* (in Upjohn units, U.U.) which was administered subcutaneously except where the figure is enclosed in a square, in which case intravenous injection (i.v.) was resorted to. Where the exact day of ovulation was known it is indicated by *O*. *M* = menses, the letter being preceded by a number representing the length of the menstrual cycle in days. The symbol for female (♀) with one line = semi-castration; with 2 lines = bilateral castration; † = killed; *L* = laparotomy for inspection only; *H* = laparotomy and hysterotomy; *P* = diagnosis made by palpation; the solid triangle = copulation. Where the result is not covered by the general heading of the table it is specifically stated.

Certain details concerning most of the experiments are contained in Table 1, those for No. 451 in Table 2, for No. 305 in Table 3, for Nos. 395, 442 and 516 in Table 4. The variety of effects possible in the same animal is well illustrated by the three experiments on No. 451 which are summarized in the following protocol:

No. 451. On Feb. 13, 1937, day 20 of the cycle, 7 days after ovulation, the ovary containing the corpus luteum was removed. The next bleeding occurred some time before Feb. 18. 60 U. U. of *Gonadogen* in the following cylce produced little if any effect and the animal remained refractory until the next autumn (Table 2).

Ovulation occurred spontaneously in the cycle of Sept. 22–Oct. 20. Oct. 30 (day 11) 20 U. U. of *Gonadogen* injected intravenously; Nov. 1, follicle in ovary diagnosed by palpation; Nov. 2 (day 13), 3 mm. foll. seen at laparotomy; Nov. 4 (day 15) ovulation diagnosed by palpation.

The next two cycles were short (20 days) and probably non-ovulatory (bleedings Nov. 17, Dec. 7 and Dec. 27). Treatment with *Gonadogen* Jan. 5–7 as shown in Table 2. Jan. 11 (day 16), colchicin administered, animal killed 10 hours later. The ovary contained two large follicles, histologically normal, except for excess of coagulum in the liquor folliculi (figs. 4 and 5). Sections of uterus, cervix, and vagina showed the endocrine effects of the ovary to be essentially normal (fig. 6).

II. NORMAL FOLLICULAR ENLARGEMENT WITHOUT OVULATION

This series of experiments includes the five cases mentioned in Table 2 and in addition No. 430 of Table 1 and No. 442 of Table 4. The following details concerning experiments on two of the animals offer special points of interest:

No. 577. This animal and the following are of interest in showing response to *Gonadogen* given in oil (See note[1] and Table 2).

No. 580. In this female a 3.5 mm. follicle was noted at laparotomy on Feb. 9 (day 11). Menses Feb. 25; cycle 26 days in length. On Feb. 25 (day 1) injection as shown in Table 2. The ovary became enlarged but ovulation did not occur. Cycle 26 days.

In the following cycle the effect of successive injections (Table 2) were checked by two laparotomies. On the third day of injection (day 10) there were no visible follicles in the ovary; 6 days later (day 16) the ovaries were removed. One ovary contained a normal 3 mm. follicle. It is not probable that ovulation would have occurred later for day 16 is rather late in the cycle for the appearance of a growing follicle occurring spontaneously.

Eight cases are cited in which one or at most two large follicles (3–6 mm. in diam.) were found at laparotomy after injection of *Gonadogen*. It is questionable how many of these would have rup-

tured and yielded fertilizable ova. In one case (No. 451) a follicle seen at laparotomy is known to have ruptured later but no such positive diagnosis was made in other cases. It is more likely that in most cases the observed follicles were sufficiently injured to render them subject to progressive atresia rather than rupture.

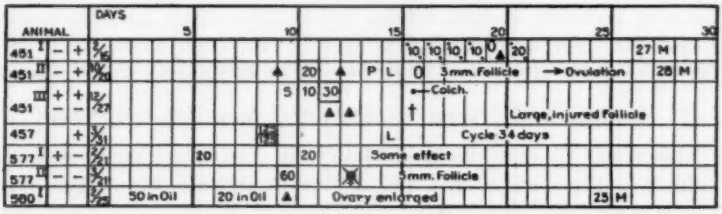
III. DIFFUSE OVERSTIMULATION OF THE OVARIES

Sixteen experiments resulted in ovaries greatly overstimulated (Table 3). Most often several or many follicles were involved, in only 3 cases a single follicle. The large variety of effects will appear from a reading of the protocols given below. In two cases the left

TABLE 2

Cases in which large normal follicles were produced

Explanatory notes under Table 1



ovary was little affected, but in all other cases the response was bilateral.

1. Far-reaching Injury to the Follicle. In No. 574, an early mature female, after a course of injections of *Gonadogen*, a laparotomy was performed at which the ovaries were found to contain numerous large, clear follicles. One ovary was removed on March 17, day 15, and sectioned (fig. 1); the follicles contained a healthy but somewhat thickened granulosa full of mitoses. Immediately after laparotomy 100 R.U. of *Follutein* was injected intravenously and the next day 50 additional R.U., subcutaneously. On March 20, 10 hours after colchicin administration, the animal was killed. The surviving ovary was found to be profoundly disrupted, as compared with its mate removed 3 days previously. Every follicle had reached a state of extreme degeneration (figs. 2 and 3). It appears that the de-

generation of the granulosa cells proceeded more rapidly than that of the ova, for well preserved ova may be seen floating free in highly degenerated follicles.

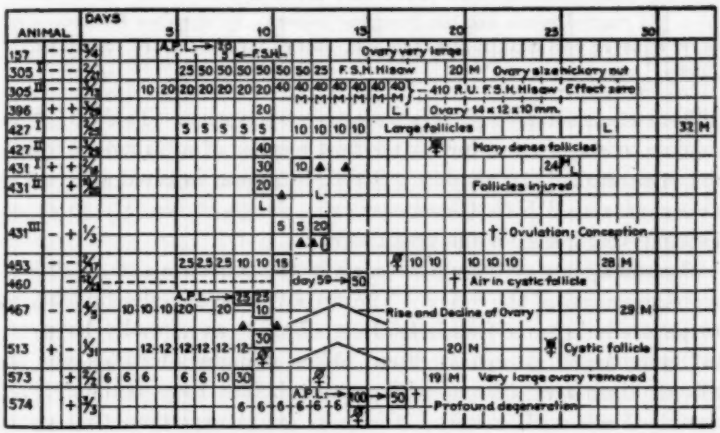
A similar fate appears to have struck the rapidly growing and involuting follicles of No. 467, as determined by palpation alone (Table 3).

Both attempts, therefore, to bring about ovulation in large follicles by excessive doses of luteinizing hormone proved disastrous, resulting merely in more profound necrosis of the granulosa.

TABLE 3

Cases in which the injections resulted in overstimulation and palpable damage to the follicles

Explanatory notes under Table 1



2. Single, Greatly Injured Follicle (Nos. 157, 305[I], 460). Of special interest is No. 460 which was treated as indicated in Table 3 and killed by air embolism. Some of the air reached the highly congested ovary and found its way into the cystic follicle (fig. 11). The granulosa was much degenerated and the uterus was in the stage of early pro-estrum, hence was less stimulated than normally at the time of ovulation. The hormone had, therefore, probably precipitated not the rupture, but, instead, the rapid involution of already atretic follicles.

3. Several to Many Enlarged Follicles.—Nos. 427[I], 427[II], 396, 430[I] (Table 1), 431[I], 431[II], 453, 513, 573, 574[I]. Several notes may be added to the information obtainable from Table 3.

No. 427 delivered a dead fetus on May 5, 1936 and furnished a 13-day ovum on November 6. Menses occurred on Dec. 11, Jan. 4, and Feb. 3, 1937, during which time a spontaneous cyst appeared in the right ovary, reaching the size of a hickory nut in December, of a hazelnut in January. Later injections caused diffuse enlargement of follicles but no cysts.

No. 513. The right ovary of this animal was removed after it was stimulated by *Gonadogen* (figs. 8 and 10). Since the follicles of both ovaries were clear and possibly "ready to rupture" 30 U. U. *Gonadogen* was injected immediately. But rapid involution, instead of ovulation, took place, as disclosed by palpation. Within 37 days after the first operation all recognizable vestiges of the large follicles had disappeared (fig. 9).

No. 573 reacted much like the foregoing animal. In this case the recovered ovary, removed two months after the first, showed no trace of the former cystic follicles.

From the protocols of this series of experiments (Table 3) it is possible to extract a recipe for the best method of injuring the follicular apparatus of the monkey. Strong luteinizer injected after overtreatment with *F.S.H.* (*Gonadogen*) was shown in No. 574 to cause a violent disruption of the granulosa.

The commonest pathological lesion in the series consists of diffuse overstimulation of the ovary. The reagent is seldom discriminative, for generally not one follicle is alone affected, as normally happens, but many begin growth at once. When numerous follicles are involved, they are stricken with atresia before they become truly cystic. Their walls attain a thickness far greater than that characterizing the normally maturing follicle (fig. 5). Long before mitosis ceases the liquor folliculi becomes cloudy with coagulable substances which in microscopic preparations are strongly eosinophilic.

Even the largest follicles can, however, scarcely be called cystic. The histology of an overstimulated follicle is quite different from that of spontaneously occurring cysts. These have been encountered a dozen times or more in the colony. They attain a maximum size equal to that of a hickory nut or walnut, and they may persist over one or several menstrual cycles. They are usually lined with flat

cells showing no necrosis, and assays of their clear fluid contents have shown them to be devoid of estrogenic substances. They do not necessarily interfere with either ovulation or menstruation. The artificially stimulated follicles, on the contrary, soon degenerate; chromatolysis of the inner layer of cells of the granulosa occurs, even while rapid cell division is still going on. Until degeneration of the granulosa is well under way, the follicle gives evidence of estrin output (No. 451) but usually the growth of the follicle is so rapid that the uterus has had insufficient time to respond as in the normal cycle.

The implication of these remarks is that under the influence of excessive doses of gonadotropic hormone a follicle or follicles grow rapidly, become injured and rapidly regress. These facts have been ascertained both by palpation (Nos. 431, 467, 305) and by laparotomies. In the case of No. 157 the huge ovary (16 mm. x 11 mm. x 10 mm.) seen at laparotomy March 14, 1935 involuted and the uterus shrank within 6 days. In No. 513 the complete disappearance, within 37 days, of maximally stimulated follicles is demonstrated by sections of the ovaries (figs. 8 and 9).

Attempts to cause rupture of follicles that had appeared normal at laparotomy, by the massive injection of additional hormone, usually proved futile (Nos. 431 and 513). The follicle in No. 431 appeared as nearly normal as in No. 442^{III}, yet the latter was made to rupture while the former degenerated.

One of the evidences of injury to a follicle is the peculiar shape and appearance of the stigma over the cystic follicle. This consists of an elongated ellipse (fig. 7), sometimes crescentic, extending over the outer surface of the follicle (Nos. 157, 430, 431^{II}). The region is thinner than the adjoining more opaque portion of the follicle, but is itself much thicker than the normal stigma just before rupture. In No. 157 there were several elevations resembling ovulation cones, which in the sheep are normally multiple. It should be stated, however, that abnormal stigmata were twice recorded in untreated animals.

It is thus seen that doses which failed to have the slightest effect on the animals listed in Sec. I played havoc with the ovaries of the animals listed in this section. Some animals were refractory at one time but responded excessively the next.

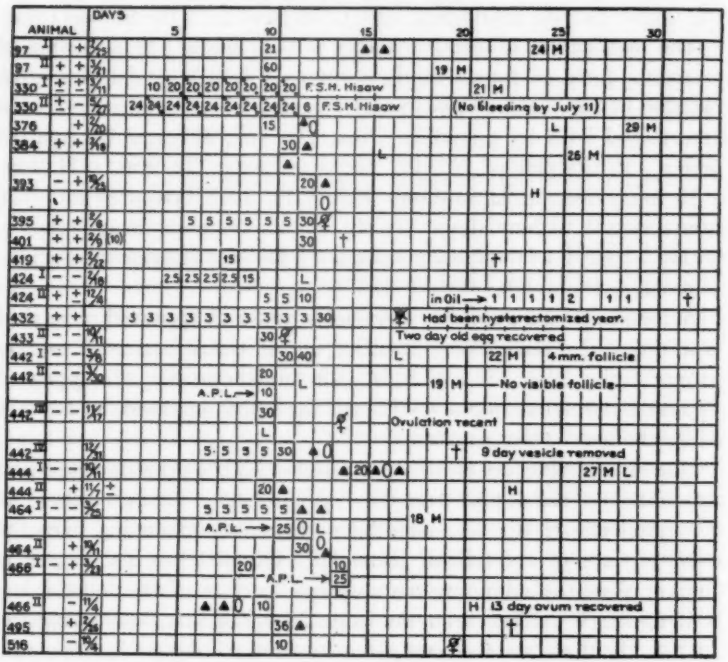
IV. OVULATION AFTER INJECTION

In this section 28 cases are included in which ovulation accompanied treatment with gonadotropic hormones. This statement is not tantamount to saying that the reagents *caused* ovulation; indeed, in

TABLE 4

Cases in which ovulation occurred

Explanatory notes under Table 1



the first five cases on the list it was proved that ovulation preceded the injections. Only in the last eight cases is the statement hazarded that the injections brought about ovulation which otherwise would not have occurred. In between are 16 doubtful cases that may be interpreted either way.

The pertinent data are summarized in Table 4. The protocols

are given in some detail because many of them constitute just the kind of controls which are difficult to obtain, and, so far, are completely lacking in women.

1. Ovulation Occurring before Injection. Nos. 395, 432, 433, 431[III], 466[II].

No. 395. The corpus luteum removed on February 19, (day 14), two days after injection of 30 U. U. *Gonadogen* intravenously, proved to be five or six days old (cf. No. 432). In the following cycle the surviving ovary of this animal was absolutely refractory to *Gamone.*

No. 431[III]. Data in Table 3. Complete recovery from overstimulation in Feb. and Oct. 1937. Ovulatory cycle Dec. 7, 1937 to Jan. 3, 1938. Mating Jan. 14 and 15 at time of injection. On Jan. 24 a free vesicle (C 613) was recovered, which fixes ovulation on Jan. 15, that is, before the injection of a significant quantity of the hormone, for it is hardly likely that the 10 units injected subcutaneously on the two days before ovulation were responsible for it.

No. 466[II]. Mated Oct. 11–12 and Oct. 12–13. As palpation had failed to recognize ovulation by Oct. 14 (day 11) 10 U. U. of *Gonadogen* were injected intravenously. Recovery of a 13-day ovum on Oct. 25, however, proved palpation to have been faulty and the injections futile.

2. Animals that would Possibly have Ovulated without Treatment. Of these twelve were ovulating before and after treatment, three after but not before. No. 237 had been amenorrhic for four months preceding the treatment; ovulation was determined by palpation 24 hours after injection and corroborated by laparotomy three days later. The animal ovulated spontaneously and conceived during the following cycle, which is also true of No. 464[I].

3. Ovulation with Reasonable Certainty Due to Injection. Fair to excellent evidence is offered by 7 experiments that ovulation was actually to be ascribed to the administered reagent. Of these 4 were not mated, 3 were mated but did not conceive and one conceived (No. 442[IV]). While the salient data are to be found in Table 4, certain cases have features of special interest included in the following protocols:

No. 256[II]. One ovulatory cycle of 28 days; accidental rupture of follicle by pressure of palpation in 24-day cycle; non-ovulatory cycle of 21 days. After production of ovulation, which was checked by laparotomy, menses occurred on day 22 and the next cycle was 20 days in length. After this the animal became refractory to *Gonadogen.*

Nos. 330 and 424 had not been ovulating when the experiment was made nor did they ovulate in the immediately succeeding cycles. No. 330 remained refractory to a second course of injections in May.

No. 442 (Complete protocol including 4 treatments with *Gonadogen* having 4 different effects).

No. 442^{I}. Acquired in May 1936. Began menstruating in September. Cycles of 35, 30, 35, 28, 33 days were non-ovulatory. 30 U. U. *Gonadogen* intravenously March 18 (day 11), plus 40 units the next day produced only a 4 mm. follicle by March 24. The cycle was 22 days in length.

No. 442^{II}. 20 U. U. *Gonadogen* plus 10 R. U. *Follutein*, day 10 of following cycle, had no visible effect on ovary inspected on April 10, but cycle was shortened to 20 days.

No. 442^{III}. The first two autumn cycles (1937) of 31 and 27 days were non-ovulatory. On November 26 (day 10) the ovaries were inspected and a 4 mm. follicle seen; 30 U. U. *Gonadogen* injected intravenously. Four days later the ovary with corpus luteum was removed. The ruptured follicle was not over a day old, hence ovulation occurred about 3 days after injection. No bleeding followed this semi-castration so far as revealed in vaginal lavages.

No. 442^{IV}. The next menses Dec. 31. Animal perceptibly in poor condition. Injections as in Table 4. Mating Jan. 11. Ovulation within 24 hours. On Jan. 19, 1937, the animal was killed and a lightly attached vesicle (C 610) was recovered from the uterus.

No. 444. Two ovulations were induced in the right ovary in successive cycles by means of *Gonadogen*.

No. 464^{I}. Cycles regular (25, 27, 25, 26, 26 days) but non-ovulatory. On April 10 (day 10) 10 U. U. *Gonadogen* and 25 R. U. *Follutein* were injected intravenously and 2 days later a freshly ruptured follicle was seen at laparotomy. Bleeding followed 8 or 9 days after ovulation; cycle 18 days.

One may well question whether the endocrine set-up revealed by this all too prompt bleeding would have been suited to the effectual implantation of the fertilized ovum. In the second experiment on this animal menstuation did not occur until 19 days after ovulation, 20–21 days after injection of *Gonadogen* which would seem to reflect a more nearly normal endocrine relation.

It is believed that the preceding 28 experiments in which ovulation occurred were subjected to sufficiently strict controls to warrant several conclusions:

1) In 5 cases it is certain that the injections had nothing to do with ovulation, since subsequent examination of the corpus luteum or the fertilized ovum proved ovulation to have antedated the injections. The recovered material, including uteri, were, however, perfectly normal.

2) 16 experiments leave an element of doubt as to whether the

demonstrated ovulation was induced or could have occurred spontaneously. In those ovaries of this series, which were studied, there was little if any congestion beyond the normal condition, which is itself subject to very wide variations. In No. 431 a valuable free vesicle was recovered in the course of the experiments.

3) There remain 7 cases in which one may say with reasonable certainty that ovulation was actually induced by the *Gonadogen* employed. It is practically certain that one 10-day ovum would not have been secured without the use of gonadotropic extracts.

SUMMARY AND CONCLUSIONS

Since the conviction is growing among gynecologists that many cases of sterility in women are due to the failure of ovulation, it seemed desirable to test the therapeutic value of the new gonadotropic substances in corresponding situations in the rhesus monkey, in which species the author has demonstrated a high incidence of non-ovulatory menstrual cycles. 104 experiments are here reported, mostly performed with the Upjohn Company's product *Gonadogen*,[1] made from the serum of pregnant mares and known to contain a high percentage of follicle stimulating hormone as compared with the luteinizer (Cole and Hart, 1930).

The most essential data of the experiments are condensed in Tables 1 to 4, supplemented by additional protocols giving facts of importance for the reader's evaluation of the results obtained. The effects of the injected substances were determined in part by palpation, in part by direct inspection of the ovaries at laparotomy. Serial sections of ovaries removed in significant experiments often served to complete and make certain the diagnosis.

The results are far from promising. In 32 cases the animals remained entirely refractory. In only half of these could the general bodily condition be held responsible for the failure to respond. The doses are quite comparable to those which in other females, and at other times in the same animal, proved adequate or even much too potent.

The cases of overstimulation number sixteen. In these the ovaries at first were fairly normal and could be shown to have secretory action; but the granulosa soon became unduly hypertrophied by excessive cell division and the *liquor folliculi* cloudy and dense. The

follicle soon became stricken with chromatolysis. In one case a month sufficed for nearly complete retrogression and the establishment of normality.

A number of follicles gave evidence of abnormal stimulation by the aberrant character of the stigmata; all cases of overstimulation were characterized by congestion of the ovary. In No. 460 the disrupted condition of the blood vessels was demonstrated by a bubble of air found in the follicle after the animal had been killed by air embolism.

Even moderately injured follicles could not be brought to the rupture point with relatively huge doses of *Gonadogen* or *Follutein* injected intravenously. *Follutein* added to *Gonadogen* caused profound disruption of the whole follicular apparatus of an ovary. Injured ovaries may recover, however, and may function normally again after a period of time.

The rapid regression of injured follicles accounts for the shortening of the menstrual cycle after treatment (Tables 1–4). Even where no effect of the injections could be noted in the ovary the cycle was frequently greatly shortened, namely, to an average of 22 days in 19 cases, including only 3 cycles over 21 days in length and one as short as 16 days. In 6 overstimulated animals the average was 23 days; in the two groups together it was 22 days. But where normal follicles were produced (which may or may not have subsequently ovulated), the average of 13 cycles was 26 days, the series including three of 18, 19 and 21 days respectively.

Growth of follicles was usually produced at too rapid a rate. Under these conditions the growth of the uterus does not keep pace, for the time factor is of importance in the response of organs to hormones, and it varies with the species. In planning injections, one should seek to imitate the normal phases of the cycle characteristic of the species used. Thus, what can be done in two days in the rat, would require ten or more days in the monkey. In other words, the proœstrus phase varies greatly from species to species, whereas the progravid phase, *i.e.*, from ovulation to nidation of the ovum, is fairly constant, generally from 7 to 9 days.

There are indications, therefore, that the production of ovulation does not guarantee an endocrine condition favorable to the implantation of a fertilized ovum, for in some cases the menses occurred too

soon after ovulation, in No. 464[1], for example, as early as the sixth day. Such cycles resemble the 5-day cycle in the rat. To insure the progravid phase necessary for nidation, the rat hypophysis produces the necessary luteinizer as a result of coitus. In the monkey cited the luteinizer was presumably insufficient for suitable endometrial changes to supervene, even though ovulation had taken place.

In 28 experiments ovulation was diagnosed as a concomitant of the injections. But a closer examination of the data served to dampen one's enthusiasm for the method. For it appeared that 5 animals ovulated before the injections were made and 16 others leave an element of doubt, since they ovulated spontaneously either before or after the treatment. There remain a total of 7 unequivocal cases in which ovulation may rightly be attributed to the injections, hence the injections may be said to have been only 7 per cent "successful," if one leaves out all of the doubtful cases. The percentage rises, however, if one bases the calculation solely upon those cases in which ovulation was not to be expected in any event.

Injured ovaries may recover completely and function again after a time, which confirms the conclusion of van Wagenen and Morse (1938) based on four convincing experiments. The protocols of the present series of experiments offer the following facts in point:

Of eight animals whose ovaries had been palpably injured, six ovulated again and of those two conceived and furnished implanted ova for the embryological collection. In two further cases the surviving ovary, removed one or two months later, was found to be essentially normal (figs. 3 and 4). Seven animals, after slight to moderate response, ovulated again and two of these furnished embryos. Besides these cases, monkey No. 444 was made to ovulate in two successive cycles, No. 464 conceived twice after one induced ovulation, but suffered one early abortion. Nos. 237 and 330 ovulated spontaneously in the cycle immediately following an induced ovulation; the former furnished a normal 110-day fetus.

It is, therefore, a justifiable conclusion that the manifestly deleterious action of the extracts employed, is temporary, and that recovery is prompt and complete. No facts have come to light which would lead one to condemn the use of gonadotropic extracts in attempts to help sterile women when they are desperately desirous of having children.

The explanation of the failures lies in the difficulty of estimating the correct dosage. For example, a mature monkey is not as near "standard" as a juvenile animal of any given age. Two healthy non-ovulating females may appear perfectly comparable as far as their general appearance and cage behavior are concerned; yet 30 U.U. of *Gonadogen* injected into the one may not have the slightest effect, while it may temporarily disrupt the ovaries of the other. One would expect the same difficulty in treating women.

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PLATE I

FIG. 1. Right ovary of No. 574 after injection of 6 U. U. of *Gonadogen* daily for 6 days (March 17, 1938). × 10.

FIGS. 2 and 3. Whole section (× 10) and detail (× 40) of left ovary three days later, *Follutein* having been injected in the meantime (see Table 3). The disruption of the tissues is apparent.

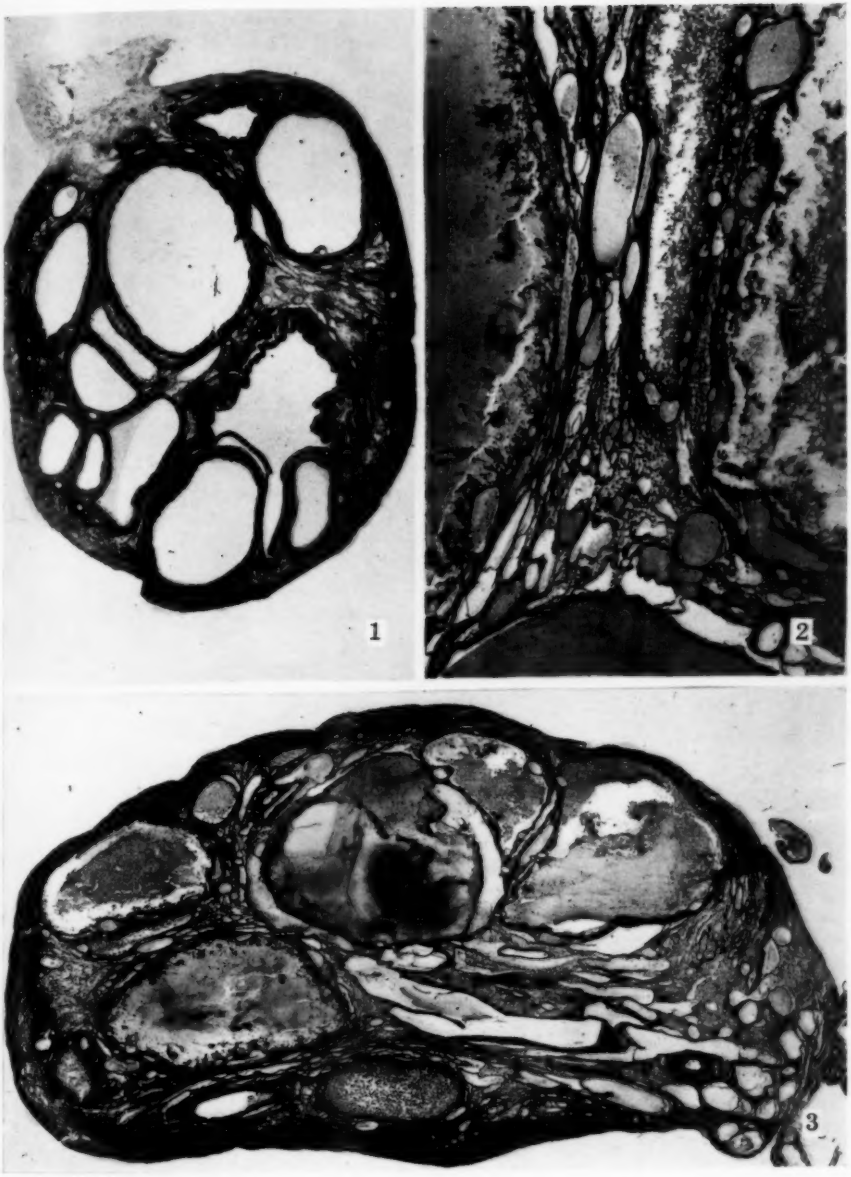

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PLATE II

FIG. 4. Large follicle in ovary of No. 451 (Jan. 11, 1938) injected with *Gonadogen* as shown in Table 3. × 10.

FIG. 5. A portion of follicle wall × 200, colchicin technique. Numerous mitoses are present in the *granulosa* membrane, a few in the *theca interna* which at this point is of unusual thickness.

FIG. 6. Uterine mucosa of same animal. × 10.

FIG. 7. Ovary of No. 430, removed after injection of *Gonadogen* (March 9, 1938). The aberrant "stigma" is well shown. × 4.

4

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6

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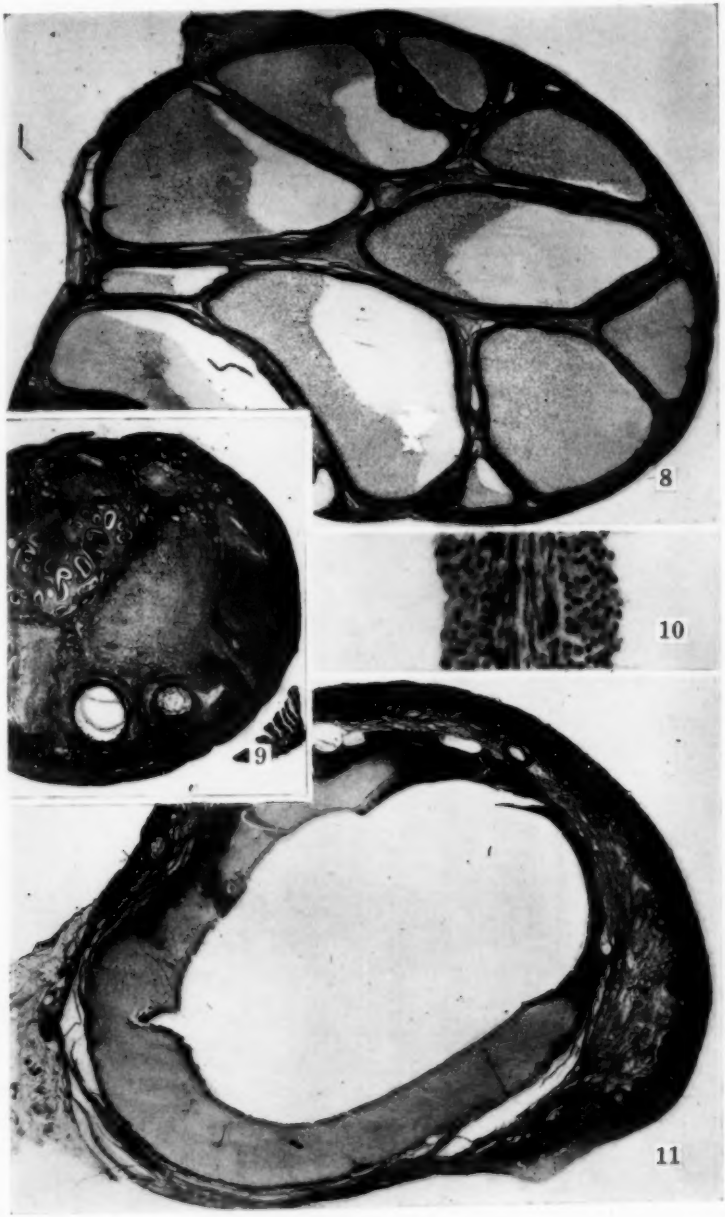
PLATE III

FIG. 8. Right ovary of No. 513 after injection of *Gonadogen* (Feb. 9, 1938). × 10.

FIG. 9. The left ovary of same animal, (March 18, 1938) showing complete recovery from cystic condition. × 10.

FIG. 10. Portion of follicle walls from section shown in Fig. 8.

FIG. 11. Air bubble in cystic follicle of No. 460, killed Jan. 8, 1938 by means of air embolism. × 10.



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RESEARCHES ON TETANUS[1]

IX. Further Evidence to Show that Tetanus Toxin Is Not Carried to Central Neurons by Way of the Axis Cylinders of Motor Nerves

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I. INTRODUCTION

It had not been our intention to devote another paper to this controversial subject, inasmuch as we believed that our earlier papers had demonstrated that tetanus toxin is not conveyed by any part of the motor nerve trunks to the reactive centers of the neural axis. Recent papers by Doerr and his associates, however, as well as certain statements made by others, have impelled us to restate our position and to give further evidence in support of it. Doerr, in particular, believes that the experiments recently made by himself and his co-workers, which will be considered later, prove that the toxin is not carried to the motor neurons of the central nervous system by way of the blood stream, but that it is absorbed by the motor nerve terminals of the voluntary muscles and is thence carried in axis cylinders to central neurons, in accordance with the well known belief of Meyer and other earlier proponents of this theory.[3] These workers assumed that the

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of observations on tetanus.

[2] Died May 26, 1938.

[3] We would cite the following references, as bearing on the point under discussion: Doerr & Seidenberg: Dynamische Aktivierung des toxisch induzierten lokalen Tetanus, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1935, **117**, 561. Topley & Wilson: *Principles of Bacteriology and Immunity*. Editorials: *Brit. M. J.*, 1935, i, 1229, and 1937, 336; *J. A. M. A.*, March 6, 1937, p. 808.

state of rigidity and contracture so characteristic of every type of local tetanus, as well as the completely analogous state of the muscles of the head and neck, the abdomen and the thorax, encountered in descending tetanus, are brought about solely by the action of the toxin on central neurons, exactly as are the tactile reflex spasms and convulsions of this more generalized type of tetanus. No satisfactory hypothesis has ever been offered to explain how a water-soluble substance of so large a molecular weight as that of tetanus toxin, once it has been absorbed by the functional motor end-organs, by naked telodendria, or by the muscle fibers of the higher vertebrates, can be transported to central neurons in axis cylinders.

The axis cylinder carriage theory assumes that the toxin is not bound by the motor end-organs or the axis cylinders, that they do not immobilize it, that it can move from point to point in them in its natural and unaltered state, and that the axis cylinders serve merely as nature's conduits for its centripetal transportation.

The Fixation of Toxin by Motor End-Organs or Other Motor Nerve Terminals Precludes the Possibility of Its Transference in Axis Cylinders to Anterior Horn Cells and Their Synapses

It cannot be doubted that *tetanus toxin induces local tetanus when brought into direct contact with voluntary muscles* by means of the hypodermic needle, and that it necessarily induces a similar state of progressively increasing rigidity when sufficient amounts of it are brought to the more susceptible muscles of the body *via* the blood stream. This implies, if we accept the pharmacological theories of the day, that the toxin produces this effect after being absorbed by and fixed upon the motor end-organs. We regard it as quite possible that any other motor nerve terminals that do not form a part of the motor end-organs, if such exist in the muscles of the higher vertebrates, will also absorb and fix the toxin. Furthermore, we regard it as quite possible that when a nerve trunk, such as that of the sciatic, is thoroughly impregnated with intraneurally injected toxin, this diffusible substance, which passes into and out of the blood and lymphatic capillaries with the greatest ease, finds its way into the axis cylinders at Ranvier's nodes, at which points no obstacle other than an excessively

thin membrane stands in the way of its entrance.[4] It must be borne in mind in this connection that in the course of a long nerve, such as the sciatic, such possible points of entrance are exceedingly numerous, since the distance from one Ranvier's node to the next amounts to about 50 microns or, in extreme instances, to 1 mm. But all of the toxin that may be transferred to the axis cylinders *via* Ranvier's nodes will soon be fixed upon them and become incapable of being moved from one point to another, with retention of its native properties, by any known forces at the body's disposal. Even if it be assumed that there is enough interstitial fluid between or around the bundles of nerve fibers to retain an appreciable amount of non-fixed toxin, it would have to be explained how this retained toxin can reach central neurons.

The rapid fixation and consequent immobilization of the toxin is well shown by the symptoms that follow the injection of so small a quantity as $\frac{1}{300}$ of a lethal dose[5] into the anterior horn of the spinal cord. Evidence of a similar localized fixation by motor end-organs, or muscle fibers, is obtained by the intramuscular injection of $\frac{1}{300}$ of a lethal dose into an extremity; the resulting local tetanus of a single extremity—a variety of tetanus that can be induced only by a topical administration of the toxin—attests the fixation. It must be recalled in this connection that, in all experiments of this kind, after time has been given for the removal of any injected excess of the toxin

[4] It is pointed out by Voss, in a recent and very excellent paper, entitled "Der Aufbau der Gewebe vom ernährungsphysiologischen Standpunkt betrachtet," that the protein-lipoid composition of the myelin sheath stands in the way of the ready diffusion of nutritive material to the axis cylinders. The occurrence in the myelin sheath at frequent intervals of Ranvier's nodes, at which points the axis cylinders are covered only by a very thin membrane, affords an arrangement by which nutritive material can enter the axis cylinders, and it may be added, that metabolic products can also escape at these points. According to the author, this idea of the importance of Ranvier's nodes for the nutrition of the axis cylinders has long met with general acceptance. This paper by Voss contains much valuable material in respect to the microscopic interstitial anatomical structures by which epithelial cells, various types of cartilage, the bones and muscle fibrils are nourished.

[5] See *Researches on Tetanus. VIII* (footnote page 610) for definition of *dog lethal dose.*

by the blood stream, no one has ever been able to demonstrate the presence of the toxin at the injected points by means of the biological assay. The presence of fixed toxin can then no longer be proved by such an assay but only by the subsequent appearance of the symptoms of tetanus, as was pointed out in *Paper V* of our series. Fixation upon receptive tissues, for the varying periods of time required for the induction of characteristic effects, holds for the great majority of physiologically active drugs and poisons. Here also repeated exsanguinations and infusions might enable us to determine the actual amount of a given physiological agent that was fixed in the body, provided that highly sensitive tests for its detection in the body fluids and secretions are available. Certainly the absorption and early fixation of tetanus toxin by the various tissues of the body, in such a manner that it can no longer be removed from them by the circulating blood or by salt solution, appears to be well established by many facts that have been given in *Papers V, VI, VII* and *VIII* of our series.

We therefore come to the conclusion that from the moment that the toxin is fixed upon motor end-organs and upon central neurons (and this fixation appears to begin immediately upon its contact with them), it has so far lost its molecular mobility that its carriage in a peripheral nerve or in the spinal cord, from point to point by molecular forces, becomes impossible. Any organ that has not yet received enough of the toxin to satisfy its fixation capacity can receive further increments only by way of the blood vascular or lymphatic system. The above argument alone suffices to show the untenability of the axis cylinder carriage theory. Mechanical forces cannot be here invoked by the adherents of the theory. It is entirely disregarded, for example, that it would probably require several months for the assumed neural transportation of freely movable toxin, by molecular forces, by way of the sciatic nerve from an infected hoof of a horse to its spinal motor neurons. "Sir W. B. Hardy once said, it would require geological time for diffusion to work between the spinal cord and the tail of a whale." (A. V. Hill: *Chemical Wave Transmission in Nerve, 1932*, page 5.)

II. THE AXIS CYLINDER THEORY IS INCONSISTENT

The theory is inconsistent for the reason that the axis cylinders of the motor nerves are believed to effect the transmission of the toxin

to central neurons and so to influence them that they become responsible, at one time, for the appearance solely of an uncomplicated local tetanus, and at another time, for the appearance of a generalized tetanus, with all the possible symptoms of the disease.

1. *Local tetanus.* All students of tetanus are now agreed that a long-lasting muscular rigidity appears when an appropriate amount of the toxin is injected into voluntary muscles, or when it is brought to them more indirectly by lymphatic net-works as the result of subcutaneous (or intraneural) injection. Every voluntary muscle of the body that can be reached by the hypodermic syringe can be thrown into a state of rigidity (the *Starre* of German writers) by amounts of toxin that are so small that not enough of it passes into the circulation to influence the neurons of the spinal cord. We have in this manner often induced in all of the four extremities of dogs and rabbits a local tetanus that lasted for many weeks; *this was never complicated by the slightest sign of a tactile reflex tetanus*, such as always appears when a sufficient quantity of the toxin has been fixed upon the anterior horn cells of the spinal cord. In accordance with the prevailing nerve carriage theory, this form of tetanus is thought to be explainable only in the following manner. Three anatomical structures are involved: (1) The motor nerve terminals of the voluntary muscles absorb the locally injected toxin and pass it on to (2) the axis cylinders, which act as conduits for its transmission to (3) central neurons; these are so affected by the toxin as to send out efferent impulses that induce a state of rigidity in the locally injected muscles. It is to be noted in this connection that the only afferent impulses that are capable of exciting the poisoned centers of a local tetanus come to them by way of the proprioceptive fibers, while tactile stimuli are entirely inoperative.

2. *General tetanus.* If, now, in place of bringing the toxin directly into contact with voluntary muscles by means of the hypodermic syringe, we inject it intravenously, it must reach the reacting central neurons *via* the same absorbing and transmitting axis cylinders that are supposedly effective in the production of local tetanus. But when the toxin is thus distributed throughout the body an entirely different type of tetanus results. All of the symptoms of a general tetanus appear in due course, instead of only one of them, as described above. In addition to generalized muscular rigidity there appears, particu-

larly in animals that are very sensitive to the toxin, an exaggerated reflex excitability, which is characterized by repeated spasms and convulsive seizures.

It has been assumed and maintained for fifty years that three experimentally separable states, (1) the topically induced muscular rigidity called *local tetanus*, (2) the wide-spread rigidity and (3) the exaggerated reflex excitability associated with general tetanus, can be accounted for only by making use of one and the same centripetal axis cylinder mechanism. But it is difficult to understand how one and the same absorbing and conducting neural mechanism can account for these different events. Meyer and Ransom were fully aware of the contradictions involved in the nerve carriage theory, and, in order to get rid of them, evolved the theory that the toxin acts on two entirely separate centers of the motor apparatus of the spinal cord. When an adequate amount of the toxin is injected into a muscle, the motor nerve terminals absorb and supposedly transmit it, *via* the axis cylinders, directly to a type of motor center that responds to it in such a manner as to bring about an uncomplicated localized muscular rigidity (*lokale Muskelstarre*). But when the toxin is distributed by the blood stream it is picked up by all of the motor nerve terminals of the body and is then carried in the axis cylinders to all of the centers of the spinal cord; it is now supposed to reach a second additional type of center, which is responsible for the reflex hyperexcitability and convulsions of general tetanus. According to these authors there occur in tetanus, "zwei zeitlich und örtlich geschiedene Processe im Rückenmark." Firor and Jonas have subjected this theory to a critical analysis in a series[6] of injections of the toxin into the anterior horn of the intact spinal cord, and have concluded that Meyer and Ransom's theory is untenable.

III. ON NERVE-BLOCKING EXPERIMENTS IN RELATION TO THE NERVE CARRIAGE THEORY

More than forty years ago Meyer and Ransom stated that when a sufficient amount of tetanus antitoxin is injected into the sciatic nerve of a guinea-pig, cat, rabbit or dog, and thereupon an equivalent amount

[6] See *Paper VI* of our series (Firor and Jonas).

or more of tetanus toxin is injected beneath the skin of the leg, local tetanus does not make its appearance either in this leg or elsewhere. These authors headed a series of experiments dealing with this point as follows: "Endangered centers of the spinal cord can be protected against tetanus toxin by blocking the conducting nerves with antitoxin." The experiments seemed to prove that intrasciatic injections of antitoxin actually established the nerve carriage theory on a firm basis.

Teale and Embleton repeated these experiments and concluded that "the injection of tetanus antitoxin into the sciatic nerve prevents the occurrence of tetanus in that leg, or at least greatly delays the onset and diminishes the severity of the symptoms." These authors furthermore state that, "although tetanus toxin ascends to the central nervous system by way of the axis cylinders of the nerves, it also to a very great extent passes up the nerves to the cord by way of the peripheral lymphatics. Blocking of the latter paths greatly delays and in some cases completely prevents the occurrence of tetanus in the part corresponding to the nerve whose lymphatic paths have been blocked."

Teale and Embleton found that the injection of tincture of iodine, egg albumen, or horse serum into the sciatic nerves of rabbits prior to the subcutaneous injection of an adequate amount of the toxin delayed the appearance of muscular rigidity for 24 to 72 hours, as compared with control animals whose nerves had not been blocked in this manner. Indeed, in one experiment intrasciatic injection of tincture of iodine "blocked" the nerve so effectively that "even after 24 hours the leg showed no sign of tetanus."

We have found that injection of 0.05 cc. of tincture of iodine or of 90 per cent alcohol into the sciatic of the rabbit or dog injures the nerve to such a degree that complete paralysis of the lower leg promptly ensues. The *intraneural injection of such substances as tincture of iodine is the equivalent of nerve section*, for the time being; muscles paralyzed by either method cannot be stiffened by the injection of toxin.[7] When toxin is injected into a leg the sciatic nerve of which has been in-

[7] The influence of neural trauma on the development of local tetanus has recently been studied by Aristovsky and Ponomarav (*Arkh. Biol. Nauk.*, 1936, xli, 125–131). These investigators point out that even the apparently innocuous intrasciatic injection of normal serum may cause a delay in the appearance of local tetanus of the corresponding leg, owing to "traumatic irritation" of the nerve.

jured, local tetanus can appear only in those muscles whose nerve supply is still intact. This point is illustrated by the following experiment.

July 29, 1937. Under ether anesthesia and with the usual aseptic technique, 0.05 cc. of tincture of iodine was injected into the right sciatic nerve of a rabbit, at a point about 0.5 cm. distal to the point of departure of the nerve to the hamstrings. As soon as the animal had recovered from the effects of the anesthetic, there was observed a paralysis of the right lower leg, characterized by the loss of active motion of the hock joint—exactly like that seen in another rabbit whose sciatic nerve had been transected.

August 16. Paralysis of the right lower leg has remained unchanged. One-fiftieth rabbit L.D. of toxin was injected intramuscularly at many points into each hind leg.

August 24. Local tetanus of left hind leg; knee and hip joints offer marked resistance to passive flexion; no stiffness of right leg.

August 27. Local tetanus of right hind leg; knee and hip joints offer marked resistance to passive flexion; hock joint flail.

September 1. Local tetanus has cleared up in both legs; flaccid paralysis of right hock joint remains.

The time of appearance of muscular rigidity following the local injection of toxin is determined by several factors, among which are the amount of toxin employed, the site and the manner of injection and the muscular activity of the animal. Even under the most favorable conditions, that is, when toxin is injected intramuscularly at multiple points, a difference of 72 hours may be observed in the time of development of stiffness of the hind legs, as shown in the following experiment:

August 23, 1937. Rabbit W-7, weight 3.5 kg. A total of 0.1 rabbit L.D. (96 guinea-pig L.D. 50's per kg.) of tetanus toxin was injected intramuscularly into the right hind leg at 41 points; 0.1 rabbit L.D. injected similarly into the left hind leg at 50 points.

August 24. No rigidity of either hind leg.

August 25. Marked rigidity of left hind leg, which is stiffly extended; z-position cannot be obtained; animal drags leg; right hind leg shows slightly increased resistance to passive flexion; no symptoms of general tetanus.

August 27. The same.

August 28. Both legs rigid; no evidence of descending tetanus.

Thus, in this experiment, in which the sciatic nerves were not disturbed, a large dose of toxin was injected into both hind legs intramuscularly at multiple points, and yet a delay of 72 hours was observed in the development of rigidity of the right hind leg as compared with the left. The delay of 24 to 72 hours in the appearance of local tetanus in the experiments of Teale and Embleton, in which the sciatic nerve of rabbits was exposed and injected with egg albumen or horse serum, is therefore not significant.

The numerous nerve-blocking experiments made by Meyer and Ransom may be classified as follows:

(A) Those in which tetanus antitoxin was injected into a sciatic nerve, with the result that it prevented the subsequent appearance of local tetanus, when this intraneural injection of the antitoxin was followed by the *subcutaneous* injection, into the same leg, of an equivalent amount of toxin.

(B) Those in which amounts of tetanus toxin, as large as 6 lethal doses, were injected intravenously into a cat, and were then followed by an injection of the antitoxin into the femoral and sciatic nerves. The animal now developed general tetanus, with the exception of the hind legs, which remained unaffected. Here again it was assumed that after the intravenous injection of a large amount of the toxin, its upward passage in the axis cylinders of the femoral and sciatic nerves was blocked by the antitoxin that had been injected into them.

(C) Those in which the antitoxin was injected intravenously or subcutaneously, and soon thereafter suitable amounts of the toxin were injected into one sciatic nerve. In every instance (three cats) the leg whose sciatic nerve had received the toxin became rigid in the course of two days or thereabouts. In a single experiment on a dog, in which the two sciatic nerves had been injected with the toxin, the blood and lymph were charged with an excess of the injected antitoxin but, despite this excess, the intrasciatic injections of the toxin were followed by marked local tetanus in the corresponding limbs.

In view of the positive results that were obtained by Meyer and Ransom and also by Teale and Embleton in respect to the ability of intraneurally injected antitoxin to annul the usual tetanic effects of subcutaneously or intramuscularly injected toxin, it is not surprising that the experiments of these authors seemed to furnish one of the most conclusive proofs of the truth of the axis cylinder transport theory.

Meyer and Ransom did not give their nerve-blocking theory the best opportunity to show its hypothetical capabilities when they injected the toxin subcutaneously into a "protected leg," as was done in the experiments summarized under (A), instead of injecting it intramuscularly. It is obvious that when the toxin is directly injected at *many separate points* into the muscles of such a leg, it is brought into immediate contact with innumerable axon terminals of the motor nerves; since these terminals are now liberally supplied with the toxin they must, in accordance with the Meyer theory, pass it on in the

axis cylinders to that part of the nerve that has been saturated with antitoxin.

In this way, the requirements of the nerve carriage theory are adequately fulfilled. But when the toxin is injected into the skin overlying the metatarsus of the protected leg, as was done in Experiment 3, described on page 374 of Meyer and Ransom's paper, one cannot be certain that the whole of it was not carried by lymphatics to the blood stream, as was the case in some of Zupnik's experiments. Believing that our method of bringing the toxin into immediate contact with the motor nerve terminals of a protected leg would either confirm the Meyer theory or fail to do so, we proceeded as follows:

Known amounts of the toxin were injected at multiple points directly into the gastrocnemius and other muscles of the lower leg of the dog, while a known excess of the antitoxin was injected, with special care to avoid leakage,[8] high up into the sciatic nerve at a point above the groove between the trochanter and the sciatic tuberosity. It is to be noted that all of the injected muscles are innervated by the sciatic nerve. This nerve was "blocked" at the point indicated by an amount of antitoxin that would have sufficed to protect the leg against six or more times the amount of the intramuscularly injected toxin.

When the nerve-blocking experiments are made in this manner the results are entirely contrary to those obtained by Meyer and Ransom. The muscular rigidity and contracture of the injected muscles of the supposedly protected leg are in every way equal to those appearing in the muscles of the nonprotected leg, which have received the same amount of toxin. The following protocol represents but one of a series of experiments all of which had a similar outcome, with the exception of two in which a minute drop of antitoxin was permitted to escape from the nerve into the adjacent connective tissue. The course of subsequent events in the latter experiments coincided with that described under (A), the lymph-borne antitoxin having successfully protected the muscles of the corresponding leg from the effects of the locally-injected toxin.

[8] The positive results of the experiments described above, under (B) and (C), can only be attributed to the escape of the intraneurally injected toxin or antitoxin into the surrounding tissues. This factor must also be considered in connection with (A).

Dog X-3. Brown mongrel, male, age one year, weight 9.1 kg.

August 13, 1937. Under ether anesthesia and with aseptic technique, the right sciatic nerve was exposed by separating the *M. biceps femoris* from the *M. vastus lateralis*. The upper part of the nerve was carefully dissected free from the surrounding connective tissue, and 0.006 cc. of a mixture of 1 part antitoxin to 2 parts sterile broth (the antitoxin employed assayed 800 units per cubic centimeter) was injected into the center of the nerve at a level about 1 cm. proximal to the greater trochanter. Barely discernible bulging and blanching of the nerve was noted following the injection. No leakage of antitoxin was observed when the needle was withdrawn. Subcutaneous tissues and skin were closed with interrupted silk sutures.

Both hind legs were shaved and painted with iodine and alcohol, and 1 cc. of sterile broth containing $\frac{1}{10}$ dog L.D. of toxin was injected at 31 separate points into the muscles of the right lower leg, exclusive of those of the foot, all of the muscles injected being innervated by the sciatic. The left lower leg was similarly injected, at 30 points. The intraneurally injected antitoxin was capable of neutralizing six times the amount of toxin injected into the right leg or three times the total amount of toxin injected.

August 14. The animal is active. No stiffness of hind legs noted.

August 15 and 16. The same.

August 17. Marked rigidity of both hind legs with increased resistance to passive flexion at both hock joints. Both knees offer slightly increased resistance to passive flexion. No stiffness of the hip joints. The gait is characterized by eversion of both hind legs and partial flexion of the toes so that only the claws come into contact with the floor. The dog falls frequently because its claws slide backward on the smooth floor when it attempts to walk. Knee jerks are exaggerated. There is superficial infection of the wound.

August 18. The dog is no longer able to stand up. When placed on its feet the hind legs give way and the animal sprawls out on its abdomen with the hind legs extended backward. The hock joints cannot be flexed beyond a right angle.

August 19. The animal is helpless. Stiffness of the hock joints is about the same. There is a definite increase in resistance to passive flexion at both knee and hip joints.

August 20. Condition unchanged.

August 21. Stiffness at hip joints is more marked. Both hips are rigid.

August 22. The same.

August 23. Stiffness is now most marked at the hock and hip joints.

August 24. The animal is as helpless as it would have been following transection of the lumbar cord. It presents a striking appearance, with normal appearing head and forepart of the body and useless hind legs. No rigidity of the front legs observed. Stiffness of the hind legs is most marked at the hock joints, which cannot be flexed beyond a right angle. Knees offer some resistance to flexion. It is difficult to evaluate stiffness of hip joints, since the animal snaps at the examiner and actively resists any attempt to flex these joints.

August 25. Condition of the animal is about the same. The wound is open. Rigidity of the hind legs tested with the animal under light ether anesthesia. Under anesthesia stiffness of the hips and knees completely disappears, while rigidity of the hock joints remains unchanged.

August 26. The wound is granulating. No change in distribution of rigidity. No evidence of descending tetanus.

August 27. Animal found dead. *Autopsy* showed bronchopneumonia. The wound was explored and infection found to extend down to the biceps muscle. No evidence of infection was found around the sciatic nerve itself.

IV. IN TYPICAL LOCAL TETANUS WE HAVE BUT ANOTHER EXAMPLE OF THE WELL KNOWN PRINCIPLE OF THE LOCAL ACTION OF DRUGS

Practical medicine has availed itself from the earliest times of the advantages that follow the local action of drugs. As usually defined the local action of a drug is that induced at the point of application; here the drug is retained and fixed for a variable period of time, and the quantity that is locally applied is generally so small that even if all of it passes into the blood no general or systemic reaction can occur. The great majority of pharmacologically active substances induce local or peripheral as well as systemic actions. The latter, however, make their appearance only when much larger amounts of a given active substance have been carried to the various organs of the body by the circulation. And, in that case, the substance, in contradistinction to its local action, gives rise, in the great majority of instances, to several systemic symptoms, the number and character of which depend upon the number and variety of the different systems of the body that have an elective affinity for it. We shall confine ourselves here to a consideration of the local type of peripheral actions which are readily induced by the direct application of active principles to accessible points, as distinguished, for example, from actions such as the muscular reflex responses that follow upon the injection of tetanus toxin into the anterior horn of the non-transected spinal cord (Firor and Jonas, *Paper VI*).

Two facts underlie the principle of local action: (a) The organ or tissue in question must be so highly responsive to the direct application of a pharmacologically active substance that but a small fraction of its lethal dose is required to bring about the desired local effect. This requirement is so frequently met with in our day that a large

number of locally acting drugs of the most diverse character can be used without danger to life. (b) As a rule, when we induce a local action of one type or another, we are inducing by a special technique and in an exaggerated form only *one* of the *several systemic actions* of the drug in question which would inevitably follow the dispersal throughout the body of much larger amounts of it.

It may be remarked at this point that the direct application of a drug for the purpose of inducing its local action may, on occasion, call forth more than one of its possible peripheral actions. Generally speaking, however, we endeavor to produce a single, striking local effect by direct application of small amounts of a substance to some peripheral point of the body.

It needs no argument to prove that the local anesthetic action of a small amount of cocain or of one of its many congeners is due solely to its peripheral absorption and fixation for a variable period of time. This holds also for the many drugs that act as myotics or mydriatics, when introduced into the conjunctival sac; for the many local anesthetics that block sensory and motor impulses when injected into a nerve trunk; for those agents that, like curari and similarly acting principles, paralyze muscles by their direct action on motor end-organs; and holds also for certain alkaloids and the derivatives of xanthine, guanidine and choline, all of which can be shown, when tested on suitable animals, to induce muscular rigidity and contracture or muscular tremors that are truly of peripheral origin. In the great majority of these instances, as was stated above, the *local action* (which is often most easily demonstrated by experiments on cold-blooded animals) also appears as *one* of several systemic actions, which are manifested only after the agents in question have been distributed throughout the body. This point is of particular interest to us in connection with our work on tetanus, since muscular contractures that are precisely similar to local tetanus also make their appearance among the considerable number of the systemic actions of tetanus toxin. Such systemic contractures are seen in the unremitting trismus and in the abdominal and thoracic rigidities of a generalized tetanus.

When local tetanus is viewed in the light of the two facts that lie at the bottom of the principle, or law, of local action it is seen that this type of tetanus is as truly an example of the local action of tetanus

toxin as are the other examples of local action that have been enumerated above. It was shown in *Paper III* of our series that so small an amount of a highly active preparation of tetanus toxin as $\frac{1}{200}$ of its lethal dose will cause an extensive local tetanus in the hind leg of a dog or rabbit, when it is injected intramuscularly at from thirty to thirty-five or more separate points of the leg. With such doses and with larger ones we have, on many occasions in the past three years, thrown all four extremities of dogs and rabbits into a long-lasting local tetanus, without inducing at the same time the slightest symptom of a tactile reflex motor tetanus and without endangering the life of the animal.

The production of a similar muscular rigidity everywhere in the body, as a part of the general or systemic action of the toxin, calls for the subcutaneous, intramuscular or intravenous injection, at one time, of a full lethal dose, that is, of two hundred times more of the toxin than was required to induce local tetanus in one hind leg.

Experiment to Determine the Amount of Tetanus Toxin Required to Induce Local Tetanus of All of the Muscles of the Body

In order to determine how much toxin is required to induce rigidity of all the striated muscles of the body we carried out the following experiment, in which the total weight of the voluntary muscles was determined approximately. Although our figure for the total weight of the muscles is somewhat too large, including as it does the tendons, the various fasciae, nerves and blood vessels, it is accurate enough for our purpose. From previous work we know that $\frac{1}{200}$ dog L.D. of toxin, when injected at multiple points into the muscles of a leg, is capable of causing a well marked local tetanus to appear in the extremity. Once the weight of all the muscles is known, we can determine by a simple calculation how much toxin is needed to bring about a "generalized" local tetanus.

Protocol of Dog M-2

A well-nourished, part police dog about 1½ years old, male.

Weight just before making the experiment = ... 10.34 kg.

Under ether anesthesia the left carotid artery was cannulated and the animal bled to death, the blood being collected in a flask.

Weight of collected blood (approximately 52% of total blood weight) = ... 534 grams

Weight of carcass immediately after death = ... 9.8 kg.

The dog was skinned and its claws were clipped off:

Weight of skin + claws =	1.14 kg.
Weight of carcass after skinning =	8.40 kg.

The skull was sawed open and the brain scooped out. The tongue, larynx, trachea, esophagus and the thoracic and abdominal viscera were removed:

Weight of brain, tongue, trachea, esophagus, thoracic and abdominal viscera =	1.75 kg.
Weight of carcass after removal of above parts =	6.71 kg.

The muscles joining the leg to the pelvis were cut through just below the inguinal ligament anteriorly and nearly flush with the external aspect of the ilium posteriorly, and the leg was exarticulated at the hip joint:

Weight of hind leg =	680 grams
Weight of rest of carcass =	6.02 kg.

The exarticulated leg and the remainder of the carcass were boiled for several hours. The bones were then picked clean and allowed to dry:

Weight of bones of exarticulated hind leg =	80 grams
Muscles alone of hind leg weighed 680 − 80 =	600 grams
Weight of rest of bones of body =	768 grams
Muscles of rest of body weighed 6020 − 768 =	5252 grams
Ratio of weight of muscles of one hind leg to weight of all of the muscles of the body = 600/5852 =	1/9.75

It has been shown that $\frac{1}{3000}$ of a lethal dose of tetanus toxin when injected intramuscularly at many separate points suffices to produce local tetanus of all of the muscles of a dog's hind leg. Since the ratio of the weight of the muscles of one hind leg to the weight of all of the muscles of the body is 1/9.75, it would take 9.75 times $\frac{1}{3000}$, or 0.05, of a lethal dose of toxin to induce local tetanus of all of the muscles of the body. It must be borne in mind, however, that the greater part of this 0.05 of a lethal dose, upon being injected intramuscularly at many points, would promptly escape into the general circulation, where it would be entirely ineffective in bringing about any symptoms of tetanus. It therefore represents an indeterminate excess over the amount that is actually needed to induce the wide-spread local tetanus under discussion. It is clear from the work of Firor and Jonas that the amount of toxin needed to poison all the central motor neurons is

very much smaller than that required to poison all of the muscles. There can be no doubt that 0.05 of a lethal dose, if brought directly into contact with the striated muscles and the central neurons, would suffice to poison completely both of these specifically reacting tissues.

V. DOERR'S DYNAMIC ACTIVATION EXPERIMENTS

In a recent paper, entitled "Dynamische Aktivierung des toxisch induzierten lokalen Tetanus," Doerr and Seidenberg have attempted to prove by means of a new type of experiment that intramuscularly injected tetanus toxin is not carried to central neurons by the blood stream, but by way of the axis cylinders of the motor nerves, and that local tetanus is therefore as truly of central origin as are the reflexly initiated and maintained convulsive seizures of the disease. Doerr, as is evident from a study of his various papers, holds that in natural tetanus the motor nerve terminals actually absorb the toxin, but without depriving it of its mobility. It is therefore, supposedly, still capable of being transferred by these terminals to the axis cylinders, in which, again, its mobility enables it to be carried in some unexplained manner to the receptive centers of the neural axis.

Doerr's argument in support of his theory of the central origin of local tetanus runs as follows: When an effective quantity of the toxin ("Dose 1") is injected intramuscularly at a single designated point into the forelegs of a rabbit, it is absorbed by the motor nerve terminals of the muscles of these legs and is thence transported in axis cylinders to the neurons of the cervical cord; responding to sensory stimuli these neurons send out efferent impulses to the toxin-injected muscles which cause them to react with the tonic rigidity of local tetanus. When, however, this dose (1) of the toxin is injected intramuscularly at one point into the thigh muscles of the two hind legs of a normal rabbit, no evidence of local tetanus is obtainable; this is therefore called the subthreshold dose for the hind legs. In order to produce local tetanus of the hind legs three or four multiples of "Dose 1" must be injected. But when, as in Doerr's *Schema 1*, the hind legs are injected with their subthreshold dose (Dose 1), at the same time that the forelegs receive this same dose of the toxin, which is so effective in their case, the two hind legs also fall into local tetanus; they are stated to become rigid at about the same time or some days later

than the forelegs. It is clearly stated by Doerr that none of the stiffened extremities ever give, at any time, the slightest signs of an associated tactile reflex tetanus.

The explanation offered by Doerr to account for this unexpected and unusual response to the subthreshold dose of the toxin is as follows: The highly poisoned motor neurons of the cervical cord, that were responsible for the certain appearance of local tetanus in the rabbit's forelegs, send down "dynamically activating impulses" to the lumbar centers, which were not sufficiently poisoned to call forth local tetanus in the hind legs without outside help. When these supposedly activating impulses, originating in the cervical cord, reached the insufficiently poisoned lumbar centers they aroused them to such a pitch of activity that local tetanus now appeared in the hindlegs.

Two assumptions are involved in Doerr's explanation of his experimental findings, one of which is of a functional neurological character (dynamically activating impulses), while the other takes it for granted that the neurons of both the cervical and the lumbar cord had actually received the toxin (Doses 1 and 4, respectively) by way of the axis cylinders. Even when a subthreshold dose was injected at one point only, into the thigh muscles, the toxin was nevertheless believed to have been carried, in this way, to the corresponding lumbar centers in an amount that was of itself entirely insufficient to induce local tetanus, but which later became highly potent in this respect, under the continued impact of the "dynamically activating impulses" emitted by the thoroughly poisoned cervical centers.

The entire absence in Doerr's locally tetanized animals of even the mildest type of tactile reflex symptoms, not to speak of pronounced convulsive spasms, is in agreement with our belief that too little of the injected toxin was carried in the motor centers and synapses of the neural axis to induce the reflex symptoms that inevitably appear as soon as the requisite minimal amount of the toxin reaches them by way of the circulation. To induce a long-lasting local tetanus in one hind leg of a rabbit, Doerr injected intramuscularly at one point his "Dose 4," which, according to our analysis of the data given by him, is the equivalent of $\frac{4}{25}$ of a rabbit lethal dose. It is difficult for us to understand why, after the injection of $\frac{4}{25}$ rabbit L.D. into the muscles of a hind leg (whereby a local tetanus is produced in 48 hours) enough of

this relatively large amount of toxin does not also find its way, *via* axis cylinders, to the anterior horn cells of the cord and thus induce symptoms of a restricted tactile reflex tetanus, after an incubation period of three or four days. As a matter of fact, it requires but a very minute quantity of tetanus toxin to produce a restricted reflex tetanus of central origin. This has been shown by more recent and as yet unpublished experiments of Firor and Lamont, who permit us to state that they have now succeeded, by an improvement in their technique, in inducing reflex tetanus of central origin without the slightest evidence of local rigidity, by the injection into the anterior horn of the lumbar cord of so small a quantity as $\frac{1}{500}$ dog L.D.

We offer the following considerations and experiments to show why we cannot accept Doerr's dynamic activation experiments in support of the axis cylinder carriage theory and the central origin of local tetanus.

Evidence against the "dynamic activation" theory of Doerr. The most efficient way of demonstrating the local action of the toxin is to make use of the method of multiple intramuscular injections. By distributing the toxin more or less evenly throughout the muscles of the hind leg of a dog or rabbit, it is possible to induce local tetanus of an entire extremity by the use of so small an amount as $\frac{1}{100}$ of a lethal dose.[9] But, even eight times this amount of toxin, injected intramuscularly at one point, may fail to evoke detectable muscular rigidity.

In all of his experiments, Doerr employed the highly inefficient method of single intramuscular injections. His failure to appreciate the true significance of local tetanus led him into difficulties at the very outset, when he attempted to determine how much toxin it took to stiffen a rabbit's front or hind leg. The great disparity between the total weight of the muscles of the front as compared with the hind leg of a rabbit must be evident to all; the muscles of the front leg of a rabbit weigh only one-fourth as much as those of the hind leg. It is significant that Doerr found that it took four times as much toxin to tetanize a hind leg as it did to tetanize a front leg; this agrees with our conception of the local action of toxin.

Local tetanus of a rabbit's hind leg was regularly obtained by Doerr and Seidenberg only when so large a quantity as 0.00004 gram of dry toxin, or (if our calculations are correct) $\frac{4}{10}$ of a rabbit lethal dose, was injected. Thus, by the method of intramuscular injection at one point only, $\frac{1}{6}$ of a lethal dose was required to tetanize a hind leg. By our method of multiple intramuscular injection, however, it is easy to tetanize an entire leg with less than $\frac{1}{10}$ of this amount.

Doerr finally settled upon 0.00001 gram of toxin ("Dose 1") as the threshold

[9] See protocols of *Paper III.*

dose for the front leg, an amount that corresponds to $\frac{1}{25}$ of our rabbit lethal dose. He found that it took not less than $\frac{4}{25}$ rabbit L.D. ("Dose 4") to stiffen a hind leg when the front legs were not injected with the toxin. When, however, all four extremities were injected with "Dose 1" ($\frac{1}{25}$ rabbit L.D.) local tetanus developed not only in the front legs but in the hind legs. In order to explain this surprising, positive response of the muscles of the hind legs, which had been injected with a supposedly sub-operative dose of the toxin, Doerr proposed his theory of "dynamic activation." (Doerr excludes on "dosological grounds" the possible carriage, by way of the blood stream, of an effective increment of toxin from the spinal centers serving the front legs to those serving the hind legs. Caudal transport of toxin in the spinal cord itself is rejected as unproved.)

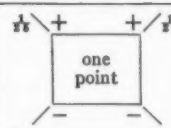
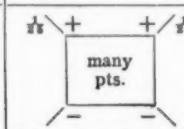
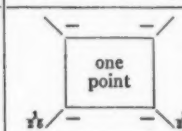
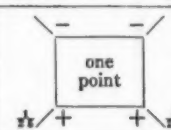
It would be unprofitable to discuss in detail the results of the various combinations of injections employed by Doerr and the deductions based upon them, such as the concept of "crossed activation." An important clue to the true significance of his results is furnished by the fact that in several of the experiments described in his paper, *dynamic activation of either the crossed or the uncrossed type failed to occur*. Professor Doerr does not give us any explanation of these negative results, which cannot be set aside as of no importance for his theory. The fundamental error that invalidates his conclusions lies in his assumption that the true threshold dose required to induce local tetanus of an extremity can be determined by the method of single intramuscular injections. His "Dose 1" ($\frac{1}{25}$ rabbit L.D.) was, of course, not, as he believed, a true suboperative or subthreshold dose for the hind leg, a fact that could easily have been discovered had he used our method of multiple intramuscular injections.

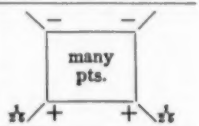
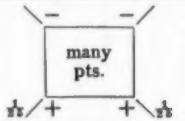
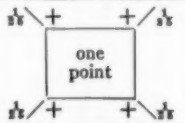
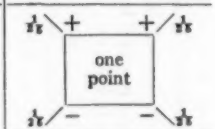
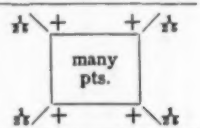
When "Dose 1" is injected at one point into the hind leg of a rabbit, local tetanus may or may not develop, depending upon the chance distribution of the toxin, either directly or by way of lymphatic capillaries, to muscles that are situated near the site of injection; if much of the toxin is deposited in the muscles, local tetanus will develop, while if most of it is carried to the venous system by blood capillaries or by the lymphatic collectors, the muscles may escape being poisoned. Thus, in Doerr's experiments, when the toxin was injected into the hind legs local tetanus developed in some instances but failed to appear in others. Our studies show that following the intramuscular injection of $\frac{1}{25}$ L.D. of toxin at one point into the hind legs, such a variation in the results occurs, regardless of whether the front legs are injected contemporaneously with an effective dose of the toxin.

In Table I are summarized a number of experiments similar to those described by Doerr and Seidenberg. In the last column of the table the results are expressed in schematic form. The rectangles represent the bodies of the rabbits and the diagonal processes, their four extremities. The figures placed at the ends of the diagonal lines represent the fraction of a rabbit lethal dose that was injected into the corresponding extremity; the plus sign indicates the development of local tetanus and the minus sign signifies a negative result. Within the rectangles is stated the manner of injecting the toxin, whether at one point or at many points.

TABLE I

Local tetanus in rabbits

DATE	RABBIT	DOSE	MANNER AND SITE OF INJECTION	RESULT	SCHEMATIC REPRESENTATION OF RESULT
9/24/37	W-9	1 L.D.	Intravenous	9/27: Malaise, tremor 9/28: Trismus, erection of ears 10/5: Dead	
10/23/37	W-21	$\frac{1}{25}$ L.D.	Intramuscular, one point, each front leg	10/25: Front legs stiff 11/2: Killed by intravenous injection of air	
10/22/37	W-19	$\frac{1}{25}$ L.D.	Intramuscular, many points, each front leg	10/24: Front legs stiff 11/2: Killed by intravenous injection of air	
10/ 6/37	W-10	$\frac{1}{25}$ L.D.	Intramuscular, one point, each hind leg	No local tetanus appeared 10/25: Killed by intravenous injection of air	
10/23/37	W-22	$\frac{1}{25}$ L.D.	Intramuscular, one point, each hind leg	10/25: Both legs stiff 11/1: Dead. No general tetanus	

10/ 6/37	W-11	$\frac{1}{25}$ L.D.	Intramuscular, many points, each hind leg	10/10: Right leg stiff 10/11: Both legs stiff 10/24: Killed by intravenous injection of air	
10/22/37	W-18	$\frac{1}{25}$ L.D.	Intramuscular, many points, each hind leg	10/24: Both legs stiff 11/1: Dead. No general tetanus	
10/23/37	W-23	$\frac{1}{25}$ L.D.	Intramuscular, one point, each of four legs	10/25: Front legs stiff 10/27: Four legs stiff 11/1: Killed by intravenous injection of air	
10/21/37	W-16	$\frac{1}{25}$ L.D.	Intramuscular, one point, each of four legs	10/23: Right front leg stiff 10/24: Both front legs stiff 10/29: Cervical rigidity. No stiffness of hind legs 10/31: Dead	
10/22/37	W-20	$\frac{1}{25}$ L.D.	Intramuscular, many points, each of four legs	10/21: Four legs stiff 11/1: Killed by intravenous injection of air	

As shown in experiments W-21 and W-19, when $\frac{1}{25}$ rabbit L.D. ("Dose 1" of Doerr) is injected intramuscularly at one point or at many points into each of the front legs, local tetanus develops within 48 hours. That the intramuscular injection of $\frac{1}{25}$ rabbit L.D. into the hind legs at only one point does not always evoke local tetanus is demonstrated by experiments W-10 and W-22. The intramuscular injection of this dose at many separate points, however, regularly calls forth local tetanus of the hind legs (experiments W-11 and W-18). When $\frac{1}{25}$ rabbit L.D. is injected intramuscularly at one point into each of the four legs (W-23 and W-16), local tetanus develops regularly in the front legs, but occasionally fails to appear in the hind legs. The multiple intramuscular injection of $\frac{1}{25}$ rabbit L.D. of toxin into each of the four extremities always induces stiffness in both the front and the hind legs (experiment W-20).

The above experiments prove that the occurrence of local tetanus in a hind leg injected with the toxin is not influenced by the injection of the toxin into a foreleg, but simply depends upon the amount of toxin supplied directly to the motor end-organs; only when a threshold dose or more is administered will local tetanus develop. It is evident that our experiments do not concur with those of Doerr and Seidenberg and so fail to support their theory of "dynamic activation."

VI. THE EFFECT OF NEUROTOMY ON THE PERIPHERAL ACTION OF TETANUS TOXIN AND OTHER POISONS

When all of the nerve trunks of an extremity or of any other part of the body are severed, subcutaneous, intramuscular or intraneural injections of the toxin are equally powerless to produce local tetanus in the neurotomized regions.[10] An easily performed experiment serves to impress this statement on the mind. The intramuscular injection of a suitable amount of the toxin at multiple points into one hind leg of a normal animal is followed by a long-lasting local tetanus, which remains confined to the injected muscles, unless more than the required quantity of the toxin has been employed; as against this result, the injection, at the same time, of an equal amount of the toxin into the muscles of the contralateral but previously completely denervated leg, is never followed by the slightest evidence of local or general tetanus.

When a lethal dose or more of the toxin is injected *per venam* into a normal four-footed animal, complete stiffness of all four of its extremities inevitably follows in due time, but it is not possible, of course,

[10] See Courmont & Doyon: "Le Tétanos," pages 11 and 12.

to induce stiffness in only one extremity by this method of administering the toxin. When all of the nerves of only one extremity are transected and a fatal dose of the toxin is thereupon administered intravenously, as before, the usual stiffness (together with the usual reflex tetanus) appears in the three legs and other parts of the body whose nerves have not been transected, while the denervated leg remains flaccid as long as life continues.

In the course of our studies on various aspects of tetanus, we have often had occasion to convince ourselves of the correctness of the foregoing statements. It is clear that toxin injected into an extremity all of whose nerves have been transected can no longer act as a poison for that particular extremity. That neurotomy leads to the disappearance of the local tetanus of human beings, provided that the rigidity be not of long duration, was well known to the internists and surgeons of the latter part of the eighteenth and the early years of the nineteenth century;[11] the operation was frequently employed as late as 1870[12] in the hope of mitigating the symptoms of the disease, but it is only within the last fifty years that earlier observations have been verified by animal experiments.[13]

These observations taken with those presently to be described are still believed by many to lend support to the axis cylinder carriage of the toxin and even to prove that local tetanus and the muscular rigidity of identical character seen in generalized tetanus are caused by the action of the toxin on central neurons and not, as we believe, by its direct action on the neuromuscular junctions. In fact, the physiological effect of nerve transections, before and after administration of the toxin, was formerly thought to preclude the possibility of the direct action of tetanus toxin on voluntary muscles. Discoveries of the past forty years relative to the manner in which peripherally acting drugs bring about their effects, whether these concern cross-striped or smooth muscles, or secretory cells, have shown, however, that nerve transections do not preclude the possibility of the peripheral action of many drugs.

The literature of pharmacology contains a number of examples of

[11] Curling; Rose.
[12] Arloing & Tripier.
[13] Brunner, Courmont & Doyon, Tizzoni & Cattani (1890).

well known substances that can be shown to act directly upon voluntary muscles with intact motor terminals but which, like tetanus toxin, fail completely to induce the usual muscular rigidity when they are injected *after* transection of the mixed nerves to muscles.

Forty years ago the pharmacologist Julius Pohl made the interesting discovery that the sodium salts of a number of halogenated fatty acids, more particularly those of monobrom-, monochlor- and dichlor-acetic acid, produce effects that are extraordinarily like those seen after the administration of tetanus toxin. These effects are best seen in cold-blooded animals, such as the various species of frogs, as is so often the case with drugs and poisons that act directly both upon voluntary muscles and central motor neurons. Of the three acids particularly studied by Pohl, in his series of halogenated compounds, the sodium salt of monobrom-acetic acid produced the most striking results in inducing muscular rigidity, or *Starre*, to use the author's term.

On injecting frogs with amounts of the sodium monobrom-acetate, varying from 0.05 to 0.0005 gm., it was observed that the larger doses began to affect muscular movements within a few minutes, while the smallest required several hours for the full development of the following symptoms. With the larger doses voluntary movements were made with difficulty, were awkward and soon became less frequent. Fibrillary muscular twitchings appeared, with now and then an extensor spasm, particularly of the hind legs. The toes were spread apart, the abdominal muscles became contracted and the muscles of the extremities, after an especially strong attack of muscular fibrillation, became rigid and hard, and this condition soon became so extreme that the animal could be raised and held up as if it had been turned into wood. The contracted muscles stood out beneath the skin as in an anatomical specimen. The head was drawn backward as a result of the rigidity of the dorsal muscles. The heart continued to beat for a time, but gradually weakened and was found to have stopped in a rigid systole or in an entirely flaccid condition.

Many years later, Lundsgaard, in the course of his classic researches on phosphogen breakdown as the energy source of muscular contraction made similar observations on the effect of iodo-acetic acid neutralized with soda upon the development of muscular rigidity both in frogs and in rabbits. Pohl had overlooked the striking muscular action of this acid (which he said gave no noteworthy symptoms), presumably because he failed to give it to frogs in the larger doses employed by Lundsgaard. Lundsgaard gives a concise description of the action of the iodo-acetate on frogs and rabbits. In the case of rabbits the intravenous injection of the neutralized iodo-acetic acid, in doses of 0.05 centigrams per kilogram of body weight, is surely fatal, and this holds also for the brominated and chlorinated acids studied by Pohl. After injection of the iodo-acetate into rabbits there occurs a latent period of 5 to 20 minutes, varying with the size of the dose, during which the rabbit appears to be entirely normal. The animal then falls

down quite suddenly, makes a few violent movements of the extremities, whereupon the entire musculature of the body falls into the most decided rigor. The thorax is fixed in a maximal inspiratory position and the animal dies for want of air. The rigid state of the entire musculature is so marked that the animal may be held by the feet in a horizontal position. In frogs the muscular rigidity continues for many hours. Lundsgaard states that he has observed frogs that remained in a state of muscular rigidity for five hours after it had become fully developed.

Pohl's description of the rapidly fatal action of an intravenously injected large dose of his sodium-monobrom-acid into rabbits is quite like that given by Lundsgaard for the sodium iodo-acetate, except that more attention is given by Pohl to the appearance, after the administration of the monobrom salt, of muscular fibrillation and exceedingly violent convulsions which soon give place to extreme rigidity of the muscles of the body, including those of the thorax.

Since in the case of the frog muscular activity can continue for many hours after the heart has ceased to act, it is quite understandable why these animals remain entirely rigid for so long a time in comparison with rabbits. Were tetanus toxin equally poisonous for the heart and the central nervous system of both cold and warm blooded animals with the halogenated acids under discussion, we could not hope to obtain either the long-lasting muscular rigidity or the long-lasting reflex symptoms that characterize both the natural and the experimentally induced tetanus of these animals.

Pohl was faced with the necessity of deciding whether the extraordinary muscular action of his halogenated acids was of peripheral or central origin. The following simple experiments were made in accordance with the usual procedure of pharmacology.

(a) The femoral artery of the right hind leg of a frog was ligated and ligatures were then passed around all of the muscles of the upper part of the thigh close to the body, with the exclusion of the sciatic nerve. This nerve was the only part of the leg that remained in functional connection with the trunk of the animal. At a certain hour, 0.025 cc. sodium-brom-acetate (a very much larger dose than was necessary) was injected, presumably into a truncal lymph sac. Fifty minutes later the entire musculature of the body, with the exception of the ligated right leg, had become rigid *"ad maximum"*; all of the joints were fixed; the right leg retained its normal flaccidity. Faradic stimulation of the right sciatic proved that it was normally excitable in inducing muscular contractions. From this experiment and many others of the same kind, Pohl concluded that the monobrom salt must be carried to the muscles of the leg in order to induce rigidity and that the muscular rigidity (*Starre*) is of peripheral origin and is not the result of an action on spinal centers. In order to decide what part of the peripheral neuromuscular apparatus is responsive to the drug, the experiment was repeated on an otherwise normal but curarized animal. The monobrom-acetate was now devoid of its usual effect — muscular rigidity did not appear.

(b) When the sciatic nerve of one hind leg of a frog was transected before ad-

ministering the monobrom salt, the muscles of the leg remained quite normal and flaccid while the rigidity appeared as usual in the other extremities. Although the muscles as well as the motor-nerve terminals of the leg whose sciatic had been transected were freely supplied with the drug, since the circulation was intact, yet there was no evidence of rigidity. Pohl next showed that in order to bring on muscular rigidity in a hitherto flaccid though poisoned leg he needed only replace the lacking efferent impulses by faradic stimulation of the peripheral end of the transected sciatic nerve. In order to demonstrate the peripherally acting agent it was necessary only to faradize the transected sciatic for from one-half to one minute with weak currents. In this way the tone of the motor end-plates was so far restored that the monobrom-acetate could exert its action and induce at once, or in the course of the next few minutes, a marked rigidity of the muscles of the lower leg.

Lundsgaard made an exactly similar experiment on frogs with his neutralized mono-iodo-acetic acid. He found, as did Pohl, that in frogs that had been curarized or deeply urethanized, no muscular rigidity appeared after injecting the iodo-acetate, so long as the frogs remained under the influence of these agents. After severing all of the nerves of a hind leg and then administering the iodo-acetate, the rigidity spread throughout the whole body, with the exception of the neurotomized, paralyzed leg. On faradizing the transected nerves of the paralyzed leg so that a short series of muscular contractions was induced, the muscles of the leg soon fell into the usual iodo-acetic contracture. The continued maintenance of this state of the leg muscles is therefore not dependent on their connection with the central nervous system. The experiments show that the normal tonic state of muscle fibers and their motor end-plates is a prerequisite to the development of the rigidity in the case of halogenated fatty acids—a circumstance that holds for a great variety of other contractures, as Lundsgaard remarks. Even so mildly acting an hypnotic as urethane so far lowered the tone of the motor end-plates that the iodo-acetate could not exert its usual action.

It is apparent from the experiments here cited that the failure of contracture-producing drugs to affect muscles deprived of their nerve supply does not justify the contention that they induce contracture by a central and not by a peripheral action.[14]

[14] A number of drugs, among which may be included acetylcholine, nicotine, physostigmine and guanidine, can be shown to act upon muscles, the nerves to which have been transected and allowed to degenerate. Such peripheral effects cannot, however, always be demonstrated directly. Guanidine, for example, induces twitching in normal but not in denervated mammalian muscle. Nevertheless, it is still possible, as Frank, Nothmann and Guttmann have shown, to demonstrate a peripheral muscular action for this drug under circumstances permitting the development of the Vulpian-Heidenhain-Sherrington phenomenon.

Does tetanus toxin belong to the class of substances that can exert their peripheral action on voluntary muscles only when the tone of their end-plates is at its best? We believe that it does. The long period of incubation that follows its injection naturally stands in the way of establishing this point experimentally in as direct a manner as that employed by Pohl and Lundsgaard in their work on frogs with the salts of halogenated acids.

Fortunately, however, as we have shown in this paper and in preceding studies on tetanus, the peripheral action of tetanus toxin can be established on a firm basis by other equally valid experimental methods.

CONCLUSION

The history of science bears testimony to the fact that it is not easy to dislodge from the mind a theory that has long been whole-heartedly accepted. We have therefore presented in this paper more arguments than might seem necessary to disprove the validity of the nerve carriage theory of tetanus toxin. Two or three positive irrefutable arguments would probably suffice to bring the majority of investigators to our point of view; others, however, more firmly convinced of the tenability of the concepts of Meyer and Marie might not accept our theory, as long as they could discover a single apparent objection to it. We have therefore reviewed critically the main arguments that have been adduced in the past in favor of the theory, and have shown that each of them may be refuted, and have given further evidence to show that tetanus toxin can be carried to the tissues of the body, whether these be the specifically reacting striated muscles and motor horn cells or structures that do not respond in a recognizable manner to the action of the toxin, *only by the same mechanisms that effect the distribution of countless other drugs and poisons*, namely, *the blood- and the lymph-vascular systems*. This poison is capable of exhibiting a dual action, the components of which are separately demonstrable by methods that have been described in foregoing papers: the first effect

Thus di-methyl guanidine hydrochloride intensifies and prolongs the contractures evoked by the injection of acetylcholine into muscles denervated seven days prior to the injection. This fact provides further proof that the irritability-augmenting influence is exerted peripherally.

is a poisoning of the motor horn cells, which is manifested solely by the appearance of reflex motor tetanus; the second results from the fixation of the toxin upon the motor end-organs of the striated muscles and is characterized by the appearance of an enduring muscular rigidity. In the general, or descending tetanus of susceptible animals, such as the guinea-pig, horse and man, both of these principal actions of tetanus toxin may be observed simultaneously. Our concept, which is quite in harmony with fundamental physiological and toxicological principles, at once simplifies the task of classifying and explaining the various types of tetanus, whether ascending or descending, local or general, without assigning to tetanus toxin a new property—the ability to travel up peripheral nerves—and without resorting to *ad hoc* hypotheses, such as the existence of dual spinal cord centers (Meyer) and the "dynamic-activation" concept (Doerr).

When all of the experimentally obtained facts that cannot be reconciled with the axis cylinder carriage theory are taken into consideration, it will be admitted, we hope, that the theory is not only untenable but entirely superfluous. We have tried to show that the facts with which the theory is not in harmony establish, among other important features of the pathology of tetanus, the true nature of tetanic rigidity as a pharmacological response to the peripheral action of the toxin.

SUMMARY

1. The chief arguments that have been brought forward in support of the axis cylinder carriage theory of Meyer and Marie have been subjected to a detailed analysis, and further evidence has been offered to show that tetanus toxin is not carried to the central nervous system by way of the peripheral nerves, but is transported throughout the body by the blood- and the lymph-vascular systems. The toxin of *clostridium tetani* is thus brought into line with countless other noxious agents for none of which has it been necessary to assume a special mode of carriage by the components of peripheral nerves. The following theory, set forth in *Paper I*, is upheld: The toxin exhibits both a central and a peripheral action, each of which may be demonstrated independently of the other. The central effect, which is characterized by reflex motor convulsions, is due to the poisoning of the motor nerve cells of the spinal cord, medulla and pons; the peripheral effect, recognized as the unremitting rigidity of voluntary

muscles, results from the fixation of the toxin upon the motor end-organs. Evidence of the rapid fixation and subsequent immobilization of the toxin is afforded by the consequences of the injection of very small quantities of the toxin into the anterior horns of the spinal cord and, by the method of multiple intramuscular injection, into a front or hind leg.

2. The defenders of the axis cylinder carriage theory have failed to explain in a satisfactory manner why it is that there appears in local tetanus but one of the two cardinal symptoms of tetanus—an enduring muscular rigidity—while in general tetanus, where the toxin can travel only by way of the same centripetal axis-cylinder apparatus that it makes use of in bringing on local tetanus, both muscular rigidity and an increased reflex excitability appear.

3. When the nerve-blocking experiments of Meyer and Ransom were repeated by us, with special precautions to avoid leakage of the intraneurally injected antitoxic serum into the surrounding tissues, and with the employment of a highly efficient method of inducing local tetanus (multiple intramuscular injection of the toxin) no protection of the extremity could be demonstrated. Local tetanus appeared in the supposedly protected leg at the same time and to the same degree as in the opposite, toxin-injected but non-protected leg, despite the fact that the amount of antitoxin given was sufficient to neutralize at least six times the quantity of toxin employed.

4. That local tetanus results from the peripheral action of tetanus toxin is indicated by the fact that it is possible to render all four extremities of a dog rigid by the multiple intramuscular injection of so small an amount as $\frac{1}{300}$ of a dog lethal dose, without the development of any signs of reflex motor tetanus. Theoretically, 0.05 dog L.D. administered in this manner would suffice to poison completely all of the striated muscles of the body.

5. A series of experiments has recently been published by Doerr, the results of which are thought by this investigator to provide additional proof of the validity of the Meyer-Marie theory and to justify the formation of a new subsidiary hypothesis, which states that the subthreshold degree of intoxication of the lumbar portion of the spinal cord, resulting from the injection of small doses of toxin into the hind legs, may be intensified by dynamically-activating impulses emanating from cervical centers that have been completely poisoned by toxin

brought to them *via* the peripheral nerves supplying the muscles of the front legs. Employing both single injections and vastly more efficient multiple intramuscular injections of the toxin, we have been able to show that the development of local tetanus in a toxin-injected hind leg is not influenced by poisoning of the front legs but depends only upon the amount applied directly to the motor end-organs; only when a threshold dose or more has been injected will local tetanus develop. The dynamic-activation concept thus lacks valid experimental support.

6. It has long been known that tetanus toxin is powerless to induce rigidity in denervated muscles and that muscles which have become rigid as the result of the action of toxin relax at once following nerve section, provided that this procedure be carried out soon enough after the appearance of the stiffness. We believe that an essential condition for the development of tetanic rigidity is normal tonicity of the motor end-organs. Neurotomy results in an immediate and sharp depression of the tonicity of the myoneural junctions, which causes these highly sensitive structures to become unresponsive to the influence of tetanus toxin. The effect of nerve transection upon the peripheral action of other poisons is discussed in *Section VI*. The observation is there made that many drugs that are powerless to affect denervated muscle are nevertheless endowed with the ability to attack normal myoneural junctions in a characteristic manner.

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CONGENITAL "ANEURYSMAL" DILATATION OF THE LEFT AURICLE

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Although enormous dilatation of the left auricle occurs not infrequently in cases of rheumatic heart disease secondary to extreme mitral stenosis, cases of localized saccular dilatation of the left auricle which develop independent of obstruction to the mitral valve are comparatively rare. Lutembacher (4, 5) has pointed out that in the latter group, the dilatation is usually due to a localized injury to the auricular wall secondary to some inflammatory or circulatory disturbance. He has described three such cases. In the first, the left auricle was adherent to the base of the right lung at the site of a caseous tuberculous abscess; in the second, there was an acute endocarditis and multiple emboli in the myocardium; in the third, although the etiology was obscure, there was an extensive pericarditis. Thus in each of these cases the "aneurysmal" dilatation was caused by a localized injury to the auricular wall. To distinguish this type of case from those in which there was diffuse dilatation secondary to mitral stenosis, Lutembacher used the term "aneurysmal" dilatation of the left auricle.

The present case differs from both of these types of left auricular enlargement in that there was neither stenosis of the mitral valve nor any evidence of localized injury to the auricular wall. There was, however, an enormous saccular dilatation of a portion of the left auricle. Because of this unusual condition, the case is reported in detail.

CASE REPORT

C. C. (H. L. H. No. A-3887) Negro girl of 5 years, admitted October 5, 1937 with the complaint of shortness of breath and palpitation of the heart of five days duration.

Family history was non-contributory.

Past history: The birth was normal; weight 7½ pounds. The feedings had been adequate and well regulated at a feeding clinic. The development was normal. The child had had measles at 4 years and pertussis[1] two months prior to the present illness. The child was not subject to colds and had rarely, if ever, had a sore throat. There was no history of rheumatic fever or chorea.

Present illness: Onset was insidious. The mother had only realized that the child was ill for five days but the child had refused to get up from bed for one week. In retrospect, the mother thought that the child's heart had been beating fast for three months and that the condition had been aggravated by the pertussis.

Physical examination: The child showed signs of severe cardiac failure. She was extremely dyspneic and orthopneic and had a severe paroxysmal cough. The face was slightly puffy and there was slight pitting edema over the sacrum and lower extremities. The chest showed no deformity.

The *heart* was enormously enlarged both to the right and to the left; the rate was extremely rapid—250 per minute. The blood pressure was 130/? (diastolic not obtained). The cardiac pulsation on the right side could be readily felt in the nipple line; on the left side in the sixth interspace outside the mid-clavicular line there was also a strong pulsation. There was a conspicuous pulsation and systolic thrill over the entire precordium. The sounds at the "apex" (*i.e.* in the sixth interspace to the left of the sternum) were similar to those so frequently heard in rheumatic heart disease. There was a systolic murmur and a pronounced gallop rhythm. The murmur was, however, not transmitted to the axilla. The heart sounds, murmurs and gallop rhythm were remarkably well heard to the right of the sternum. Over the body of the heart there was both a systolic and a diastolic murmur. The ease with which the heart sounds could be heard excluded a pericardial effusion. The sounds and murmurs were clearly different from any cardiac phenomena usually observed. *Fluoroscopic examination* showed the heart to be enormously enlarged both to the right and to the left. In striking contrast to the ease with which the cardiac impulse could be palpated over the entire precordium, the pulsations of the heart were remarkably feeble. On rotation of the child into the two oblique positions, the heart appeared enlarged in all directions. There was, however, no demonstrable displacement of the esophagus.

The respirations were extremely rapid—50 to 60 per minute. The lungs were filled with râles. In addition, there was dulness at the base of the left lung; the breath sounds were harsh and expiration was prolonged. The liver was engorged and extended down to the level of the umbilicus. The extremities, aside from the edema, were negative; there was no clubbing or cyanosis.

Laboratory examinations showed the following significant findings: The red blood cell count was 4,500,000; hemoglobin, 76 per cent; white blood cell count,

[1] The other children in the house had pertussis at the time.

16,000 with 62 per cent adult polymorphonuclear cells. The *urine* showed a heavy trace of albumin and many hyaline casts. The *Wassermann* reaction of the blood was negative. The *sedimentation rate* of the red blood cells was normal. The first *electrocardiogram* showed a rate of 245 per minute; the P-waves were not clearly visible; the T-waves were of low amplitude in Lead 1 and inverted in Leads 2 and 3; and there was an apparent right axis deviation. The second electrocardiogram (taken after digitalis) showed a rate of 200 per minute with a small inverted P-wave in Lead 1 and an upright P-wave in Lead 2. The extremely rapid rate suggested the possibility of an ectopic rhythm and, therefore, the inversion of the P-waves in Lead 1 was not considered indicative of a dextrocardia.

Clinical impression: Congenital malformation of the heart. The nature of the malformation was not clear. The possibility of an interauricular septal defect was considered because of the extreme cardiac enlargement to the right and the left, combined with a possible ectopic rhythm. The physical signs were, however, quite different from any of the previous cases of interauricular septal defect (6).

Course in the hospital: The patient remained critically ill. It was striking that as the child lay propped up in bed, instead of turning to the left as children suffering from cardiac failure so commonly do, she preferred to lie upon her right side. Digitalis, morphine, *Salyrgan* and hypertonic glucose were of no avail. The child died forty-eight hours after admission.

Autopsy No. 15532. The body was that of a thin colored female child. It weighed 22 kg. and measured 150 cm. in length. The only significant external finding was the slight edema of the face and ankles.

Each of the serous cavities contained a small amount of straw-colored fluid,—the pericardial cavity, 80 cc., each pleural cavity, 250 cc., and the abdominal cavity, 400 cc.

The chief interest centered in the heart which was enormously enlarged. Before the pericardium was opened, the cardiac borders extended 7 cm. to the right, and 10 cm. to the left of the mid-line. When the pericardium was opened, the heart presented a most extraordinary appearance (Fig. 1). There was an incomplete dextrocardia. The entire left side of the pericardial cavity was occupied by the left auricle. It was tremendously distended; its anterior surface measured 6 x 10 cm. It was so large that it compressed the lower lobe of the left lung. The apex of the heart pointed to the right and downward. The major portion of both ventricles lay entirely to the right of the mid-line. They were rotated so that the left ventricle lay in the middle and the right ventricle to the extreme right. The right auricle was concealed behind the right ventricle. The pulmonary artery lay anteriorly, both above and to the right of its normal position. The aortic arch occupied a more transverse position than normally.

Upon opening the heart, it was seen to be greatly hypertrophied. The vena cava entered the right auricle in the normal fashion. The foramen ovale was completely closed. The right auricular wall was perhaps a little thickened. There was slight but definite thickening of one of the cusps of the tricuspid valve. The internal circumference of the tricuspid valve was 11.0 cm. The chordae

tendineae were delicate. The right ventricle was dilated and its wall was hypertrophied; it measured 0.6 cm. in thickness. The cavity of the right ventricle measured 10.0 cm. from the pulmonary ring to the apex. The pulmonary leaflets were

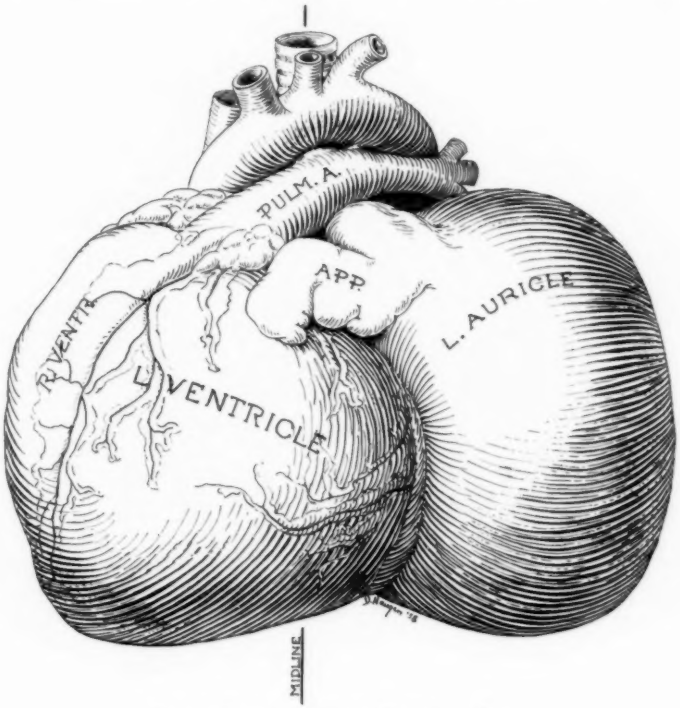

FIG. 1. SKETCH OF THE HEART IN SITU

Drawing of the heart as viewed from in front. The left auricle lay to the left of the sternum and was dilated in saccular fashion to form the left border of the heart. The apex pointed downward and to the right. The pulmonary conus lay anteriorly; and the aortic arch, transversely.

delicate; the internal circumference of the pulmonary valve was 5.0 cm. Above the valve, the artery showed no sclerosis; it measured 4.0 cm. in circumference.

The lumen of the left auricle was greatly enlarged. On its posterior aspect there was a sharply demarcated saccular dilatation which was 10.0 cm. in its greatest diameter. The wall of this greatly dilated sac was very thin and fibrous; it contained only a few criss-crossing muscle fibers. The left auricular appendage

was not dilated. The mitral valve measured 9.5 cm. The leaflets, especially the anterior one, were greatly thickened along the line of closure. The chordae tendineae were shortened and thickened.

The left ventricle, compared with the right, was small. The wall was 1.2 cm. in thickness. There was no gross scarring. Careful study of the ventricular musculature showed that the deep muscle bands lay in normal relation to each

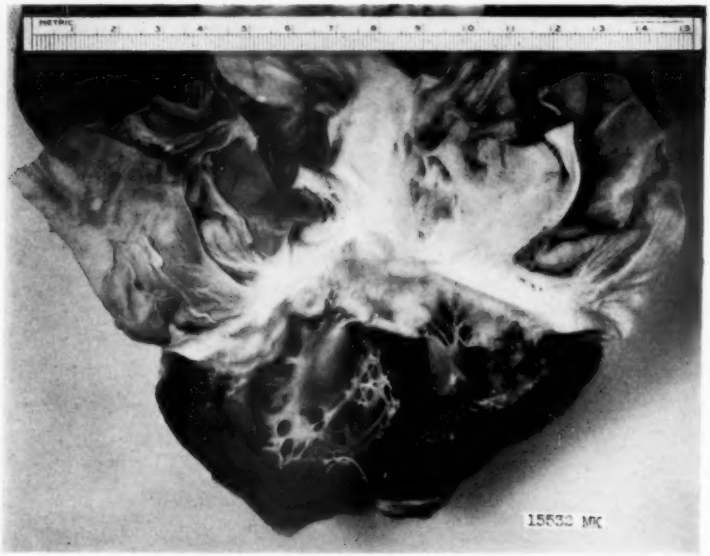

FIG. 2. POSTERIOR VIEW OF LEFT AURICLE AND VENTRICLE

The saccular dilatation has been divided in opening the heart. Its rounded edges are best seen on the right. The chordae tendineae are greatly shortened; the edges of the mitral leaflets are thickened. (Photograph by Mr. Milton Kougl.)

other. The aortic leaflets were delicate. The aortic ring measured 4.5 cm. The ventricular septum was normally formed.

The coronary arteries were smooth. Upon close examination, the circumflex branch of the left coronary artery appeared extremely small, and it was not visible where it approached the region of the saccular dilatation of the auricle.

The aorta was elastic. The ductus arteriosus was palpable as a fibrous cord. The aorta above the valves measured 4.0 cm. in internal circumference. Beyond the depression of the obliterated opening of the ductus, its circumference measured 3.8 cm. and in the abdominal portion, 3.0 cm.

The surfaces of the lungs were smooth, firm and pink. The left lower lobe was compressed and displaced upward. There was an infarct in the anterior portion of the right middle lobe. There were a few scattered patches of consolidation.

The liver was diffusely scarred on its external surface. The lobulations were distinct, with prominent central zones.

The spleen was firm with very prominent Malpighian bodies.

The other organs—tonsils, gastro-intestinal tract, kidneys and pelvic organs showed nothing abnormal.

Microscopic examination: One section of the heart included parts of the left ventricular wall, the mitral valve and ring, and the left auricular wall with its saccular dilatation. The ventricular wall was thicker than normal. The endocardium and epicardium were normal. A few round cells were seen in the perivascular tissue surrounding two of the arteries. No other changes were seen in the blood vessels. The leaflet of the mitral valve was increased in thickness; it consisted of avascular fibrous tissue. The wall of the left auricle was thinner than that of the right auricle. It contained muscle fibers and connective tissue. In the region of the saccular dilatation, muscle fibers were very few and, in some fields, completely absent. The elastic tissue fibers, stained by MacCallum's technique, were seen to be more elongated and thinner than those of a normal left auricle. Six sections taken from various portions of the myocardium showed no evidence of an inflammatory reaction.

Sections from both lungs showed pigment-containing mononuclear cells in the alveoli. A sharply delimited group of alveoli filled with red blood cells, suggested a fresh infarct.

In the spleen the venules were engorged with red blood cells. The Malpighian bodies were normally formed.

The gall bladder showed round cell infiltration in the muscular layers and around one of the arterioles in the submucosa.

In the liver the hepatic cells near the efferent veins contained fat vacuoles.

The kidneys and pelvic organs showed no lesions.

The *final anatomical diagnosis* was: Congenital sacular dilatation of the left auricle; thickening of the mitral and tricuspid valves; cardiac hypertrophy. Chronic passive congestion of lungs, liver and spleen. Pulmonary infarcts in the right middle and the left lower lobes. Slight chronic cholecystitis.

DISCUSSION

In brief, the autopsy revealed that the entire area of cardiac dulness to the left of the sternum was due to the enormously dilated left auricle. The ventricles were displaced and rotated to the right in such a manner that the apex of the heart pointed to the right and downward. The mitral valve was shortened and the chordae ten-

dineae were thickened, but there was no evidence of an acute or chronic endocarditis.

The abnormal position of the heart readily explained the unusual clinical findings. The strong cardiac impulse to the right of the sternum was due to the ventricular contraction. That the heart action had also been so easily palpable in the sixth interspace to the left of the sternum beyond the mid-clavicular line is indeed remarkable. It must have been due to the transmission of the impulse of the ventricular contraction through the dilated mitral orifice against the wall of the left auricle. This would also account for the discrepancy between the ease with which the heart beat was felt, and the feeble pulsation seen in the fluoroscopic examination. The fact that the cardiac impulse was felt further to the left than to the right explained our failure to recognize the dextrocardia. Owing to the abnormal position of the heart, the usual physical signs of left auricular enlargement were absent. It is, however, interesting to note that two of the three[2] clinical symptoms so frequently associated with enlargement of the left auricle were present, namely, tachycardia and extreme dyspnea. Had the heart occupied its normal position, the signs of left auricular enlargement would have been more conspicuous, but the findings would have closely resembled those of rheumatic heart disease.

The question immediately arose as to the cause of this extraordinary condition. At first sight it seemed conceivable that the enormous dilatation of the left auricle was due to a severe rheumatic infection and that the apex of the heart had been displaced and rotated to the right by the tremendous size of the auricle. Although the mitral valve was thickened and the chordae tendineae were shortened, there was no stenosis of the mitral orifice, and neither in the gross appearance nor in the microscopic sections was there any evidence of acute rheumatic fever or of any other infection. The changes in the mitral valve and in the chordae tendineae resembled most closely those which occur not infrequently in association with congenital malformation of the heart. Thus it seemed improbable that the abnormality of the mitral valve had caused the dilatation of the left auricle.

[2] The three clinical symptoms which frequently occur are dyspnea, tachycardia and cardiac arrhythmia.

Careful examination of the left auricle showed that its enormous size was due to a tremendous saccular dilatation which ballooned out from a sharply demarcated ring in the auricular wall. The left auricular appendage was of normal size. The fact that the auricular appendage did not share in the enlargement clearly indicated the dilatation was not a generalized process but was due to some local weakness in the muscle wall. Careful histological study revealed no evidence of any inflammatory process in the myocardium. The only abnormality found was in the caliber of the left circumflex coronary artery which supplied this area; this artery was abnormally small. Whether the decrease in the size of the artery was the cause or the result of the aneurysmal dilatation could not be determined with certainty. The small size of the artery may have been the primary condition; the defective blood supply may have interfered with the nutrition of the muscle and thereby caused the weakness in the auricular myocardium. On the other hand, the reverse of this is more in accord with the current embryological conceptions, *i.e.*, the small caliber of the artery may have been secondary to an abnormality of the auricular myocardium. In either event, the small size of this vessel suggests that the condition was due to a congenital defect either in the blood supply or in the auricular myocardium.

A review of literature failed to reveal any case of similar etiology. The findings in our case clearly indicate that it belongs in the group classified by Lutembacher as "aneurysmal" dilatation of the left auricle. In addition to the three cases reported by him, to which reference has already been made, only three additional cases of this kind have been reported.

In 1899 Lafforgue (3) described a case of congenital syphilis with an aneurysm of the left auricle which was "the size of a walnut." Bramwell and Duguid (2) in 1927 reported two cases. Both showed fibrosis of the auricular wall. In one (1), there was a rheumatic endocarditis and a chronic pericarditis; in the other (2), the cause of the fibrosis was not known. The salient features of these six cases are summarized in Table I. In all of these cases, the aneurysmal dilatation of the auricle occurred secondary to some local injury to the auricular wall. In each instance it was an acquired condition. In only one of these cases was any mention made concerning the blood supply to the

TABLE I

Summary of cases of saccular or "aneurysmal" dilatation of left auricle

CASE	SEX	AGE	DESCRIPTION OF LEFT AURICLE	MITRAL VALVE	POSITION OF LEFT AURICLE IN SITU	CORONARY VESSELS	ETIOLOGICAL DIAGNOSIS
I Lutembacher (4)	F	50	Pocket-like dilatation with traces of muscle fibers in its wall. Volume 400 cc.	Cusps adherent; rigid valvular ring	Adherent posteriorly to caseous abscess in right lung	Not described as abnormal	Tuberculosis-tubercle bacilli stained. History of rheumatic fever
II Lutembacher (5)	M	18	Dilatation began slightly above a-v groove; devoid of muscle; thick adherent pericardium; multiple infected coronary emboli; adherent to right lung. Volume 250 cc.	Admitted 3 fingers	Formed right border of heart	Perivascular lymphocytic infiltration; multiple thromboses	Acute mural endocarditis
III Lutembacher (5)	M	18	Pocket-like dilatation, 4 cm. deep; wall thin, fibrous, densely adherent to pericardium. Volume 500 cc.	Admitted 4 fingers; edges slightly thickened	Formed right border of heart	Not described as abnormal	Fibrosis secondary to unidentified inflammatory reaction
IV Bramwell and Duguid (2)	M	25	Right half dilated, thickened, adherent, almost devoid of muscle fibers; left half hypertrophied. Volume 1080 cc.	Admitted 5 fingers; leaflets were large, otherwise normal	Dilated portion above and behind right auricle	Normal	Fibrosis of auricular wall

V Bramwell and Cowan (1)	F	27	Admitted two closed fists; small "aneurysmal" pouch whose wall was devoid of muscle; scattered areas of pericarditis	Thickened, narrowed "buttonhole" type of orifice; fresh vegetations	Not described as abnormal	Normal	Rheumatic endocarditis; chronic pericarditis
VI Lafforgue (3)	M	18	Contained an aneurysm the "size of a walnut", adherent to pericardium. Gumma 16 mm. in diameter at point of adhesion	Normal	Normal	Normal	Congenital syphilis
VII Authors' case	F	6	Saccular dilatation 10 cm. in diameter; thin wall; muscle absent in some areas	Edges and chordae tendineae short and thick	Formed left border of heart	Atresia of left circumflex	Congenital malformation

affected area; in that case[3] there was perivascular round cell infiltration.

Additional and important evidence that the auricular dilatation in our case represented a congenital malformation is derived from consideration of the position of the heart. In all the other cases of enormous dilatation of the left auricle reported in the literature, the auricle either has been normally situated or has extended to the right of the sternum, and the ventricles have occupied their normal positions. In our case the aneurysmal dilatation of the left auricle occurred in conjunction with an incomplete dextrocardia.

SUMMARY

A case of enormous saccular dilatation of the left auricle in a 5 year old child is recorded. The condition is believed to have been due to a congenital abnormality either in the blood supply of the affected area or in the auricular myocardium. The thickening of the mitral valve and shortening of the chordae tendineae are believed to have been associated with the congenital malformation of the heart. The fact that the enormous saccular dilatation of the left auricle extended to the left and that it occurred in conjunction with an incomplete dextrocardia offered strong evidence that both conditions represented congenital anomalies.

The literature concerning "aneurysmal" or "saccular" dilatation of the left auricle is reviewed. The case here reported is the only one in which the dilatation of the left auricle is believed to have been due to a congenital abnormality.

The authors wish to express their gratitude to Miss D. Haugen for the drawing of the heart.

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[3] Lutembacher's case No. 2.

TUBERCULOUS ENDOCARDITIS OF THE PULMONARY VALVE

Case Report

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Submitted for publication September 27th, 1938

Tuberculosis of the endocardium, and especially of the valves, occurs infrequently. Involvement of the endocardium by spread from a focus in the myocardium, is not so uncommon, but the occurrence of isolated valvular vegetations, of definite tuberculous nature, has been proven in only four cases according to Baker (1). Since then Davie (2) has reported two cases which he regards as fulfilling the criteria necessary for diagnosis. In Baker's summary of twenty-four cases from the literature, he concludes that many of the cases referred to as tuberculous endocarditis, are not truly so, but have valvular endocarditis due to other causes. Many of these were assumed to be tuberculous because of associated massive tuberculosis. Others presented data insufficient to allow any conclusions.

The following is the only case of a tuberculous vegetation occurring on the surface of a heart valve in 15,800 autopsies performed at the Johns Hopkins Hospital. Endocardial tubercles elsewhere occurred in a number of cases. The present case is of interest, because of an isolated large polypoid tuberculous mass on the pulmonary valve, in which all of the necessary diagnostic criteria were observed.

Clinical abstract: R. W. J., a 6-year-old colored boy, was brought to the Harriet Lane Pediatric Dispensary November 2, 1937, complaining of "cough, fever, and not doing well." Three siblings showed old calcified mediastinal lesions, but no active tuberculous contact was found. Little was known of the past history. The present illness began with an attack of whooping cough about seven months before admission. The cough was severe; it continued up to the time of admission and was accompanied by considerable fever. The right ear began to drain four months

before admission. When brought to the hospital, one month before admission, because of fever, cough, and failure to do well, he was found to have a widened mediastinal shadow, on fluoroscopy. A tuberculin test with 0.01 mgm. was definitely positive. X-rays showed diffuse soft infiltration of both lungs. Shortly after this it was said that the child had had a paralytic stroke, involving the right side of the body. However, no evidence of hemiplegia was found upon examination in the dispensary. He grew rapidly worse, and was admitted to the hospital with a diagnosis of miliary tuberculosis.

His temperature on admission was 38.6°C., pulse 140, respirations 60. He was an acutely ill, emaciated, colored boy. Purulent discharge was profuse from both ears. The percussion note was hyperresonant over the chest, and many fine crackles were heard on auscultation. A soft, blowing, systolic cardiac murmur was heard; it was loudest at the second left interspace but was also transmitted far into the axilla and to the apex of the heart. The pulse was rapid and regular. The spleen was two fingers' breadth below the costal margin. The liver extended to the umbilicus. The reflexes were hyperactive.

Laboratory data: Hgb. 62%; R.B.C. 4,050,000; W.B.C. 8,000, with 86% polymorphonuclear leucocytes. A blood smear showed anisocytosis, poikilocytosis and achromia. The urine showed 2+ albumen, 2+ bile, and diacetic acid.

Course in hospital: The patient became progressively worse. Jaundice of the sclerae became evident. A diagnosis of tuberculous meningitis was made. He died on the sixth day in the hospital. Post-mortem spinal puncture showed a clear xanthrochromic fluid with 2+ globulin (Pandy), and 175 W.B.C. per cubic mm., all mononuclear cells. No organisms were seen in smears. Post-mortem blood culture was negative.

Autopsy no. 15609: The autopsy was performed 18 hours after death, on December 7th, 1937.

The peritoneal surfaces were smooth. There were dense pleural adhesions on the left side. There were 30 cc. of clear, yellow fluid in the right pleural cavity, and 75 cc. of turbid fluid in the pericardial sac. There were large caseous lymph nodes at the hilum of the left lung, and smaller nodes with no caseation at the hilum of the right lung. The cervical and mediastinal lymph nodes were enlarged and caseous. The primary tuberculous focus was apparently a caseous, partly calcified area near the hilum of the left lung. Both lungs were diffusely riddled with small tubercles. These were larger and denser at the apices. There was an intimal tubercle, covered with endothelium, protruding into the lumen of a vein in the left upper lobe. There was an old peripheral caseous nodule in the right lower lobe, but this was not calcified.

The heart weighed 130 grams. The epicardium was smooth. The right auricle was slightly enlarged; the right ventricle was distinctly large. The tricuspid valve was normal. Projecting towards the ventricular side, from the right cusp of the pulmonary valve (fig. 1), there was a cone-shaped mass, 1.2 cm. in length and approximately 3 mm. in diameter. This was yellowish, and had a roughened surface in places, but was smooth elsewhere. The free margin of the cusp was

uninvolved. The nodule extended to the base of the valve. The pulmonary artery above the valve was normal, and there were no implantations on the endocardium below. The other chambers and valves were normal.

The liver and spleen were enlarged and contained many small tubercles. The surfaces of the kidneys had many superficial, depressed, red scars. There were minute opacities which suggested tubercles. The mesenteric nodes were enlarged, but not caseous. In the lower part of the ileum, there were two small tuberculous ulcers with rolled margins, undermining the mucosa. The other organs showed no change. The fifth lumbar vertebra was caseous, but the dura was uninvolved.

The brain was large and the meninges clear. There was a firm yellowish mass protruding from the surface of the left frontal lobe, and another from the inferior surface of the right frontal lobe. On section there were a great many large caseous tubercles, sharply circumscribed and apparently encapsulated. They varied from 1 cm. to about 2.5 cm. in diameter, with many smaller ones, less than 1 mm. in diameter. In almost every section there were additional tubercles. One projected into the left lateral ventricle, but was encapsulated and there were no ependymal tubercles. A very large tubercle occupied the pons. The ventricles were not dilated.

Microscopic examination: A calcified tuberculous mass near the hilum of the left lung was thought to be the primary focus, for this was the oldest lesion which could be found, and the lymph nodes at the hilum of this lung were large and caseous. There was an old caseous nodule in the periphery of the right lung, but the lymph nodes at this hilum were not visibly changed. There were caseous nodules projecting into the lumina of branches of the pulmonary vein; these were covered over by connective tissue and endothelium.

Miliary tubercles were found in the viscera. There were caseous foci in the vertebrae, and tuberculous ulcers in the ileum. In the brain there were numerous large encapsulated tuberculomata. These for the most part had adequate capsules. Several tubercles projected into the subarachnoid space, still encapsulated by granulation tissue and fibrosis. There was a localized tuberculous meningitis over such nodules, with an exudate of plasma cells, macrophages, lymphocytes, and polymorphonuclear leucocytes, but the change was not widespread.

The pericardium showed no evidence of tuberculosis, and the smear and culture of the pericardial fluid were negative.

The polypoid vegetation on the pulmonary valve (fig. 2) was composed of typically tuberculous tissue. Step sections were studied and they may be summed up as follows: The core of the vegetation was caseous for the most part. The caseous material extended to the apex of the vegetation in some sections. Here it appeared to have broken through the endothelial covering, which was otherwise intact. The break in the endothelium corresponded to the roughened areas described in the gross. There was no adherent thrombus material, but occasionally a few cells and a few strands of fibrin. The peripheral zone and the base of the vegetation were composed of strands of epithelioid cells intermingled with lympho-

cytes and occasional giant cells. There were well formed tubercles with giant cells, radiating epithelioid cells, and encircling lymphocytes. These were found most frequently at the base of the vegetation. There was no evidence of involvement of the underlying myocardium and it was clear that the process involved the pulmonary valve as an isolated polypoid vegetation. This conclusion was confirmed by the use of Weigert's elastic tissue stain. The Kinyoun acid-fast stain showed tubercle bacilli clumped in great numbers below the apex of the vegetation and less numerous ones at other points in the peripheral granulation tissue (fig. 3). No other bacteria were found. The heart blood postmortem contained no organisms by smear or culture. The myocardium was normal, and no Aschoff bodies were present.

SUMMARY

A case of miliary tuberculosis is described in which there was a distinct tuberculous polypoid vegetation limited to a leaflet of the pulmonary valve. The histological appearance of the vegetation was characteristic of tuberculosis, and tubercle bacilli were present in great numbers. There was no evidence of involvement of the myocardium or endocardium by any other process.

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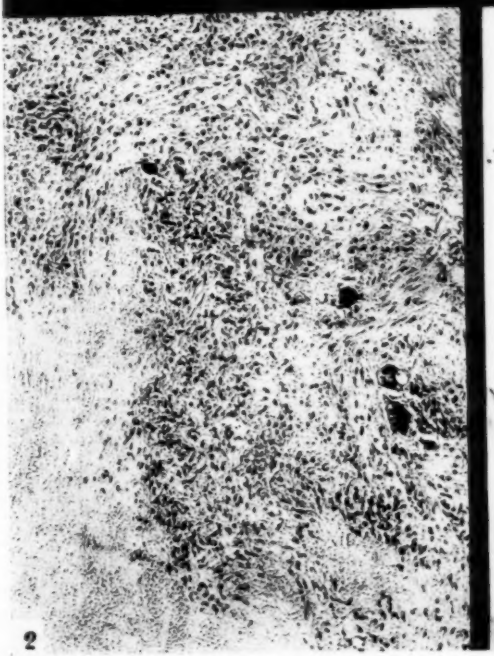
PLATE 1

(*Photographs by Mr. Milton Kougl*)

FIG. 1. Arrow points to tuberculous vegetation on leaflet of pulmonary valve.

FIG. 2. Section of vegetation showing areas of caseation with neighboring giant cells and epithelioid cells.

FIG. 3. Masses of tubercle bacilli near apex of vegetation (acid-fast stain).



UNIV. OF MICH.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.

The Chemistry of the Brain. By IRVINE H. PAGE. 444 pp. $7.50. (*Charles C. Thomas, Springfield and Baltimore, 1937.*)

Dr. Page has reviewed several topics of biochemistry that bear upon the chemistry of the brain. In his preface he states that the rôle of the lipids is emphasized. To be sure, four chapters bear upon this but most do not. The various topics are presented as if the author were attempting a well rounded exposition of brain chemistry. An introductory chapter gives a welcomed account of Thudichum but tails off with a mere bibliography in developing the "genesis of brain chemistry." Quastel has written the chapter *Oxidation in the Brain*, and Page the last chapter, *The Brain and Thought*.

The appearance of the book is, on the whole, excellent. There might have been better coöperation between author and printer in dealing with the topography of structural formulas. Some formulas would be ambiguous to unfamiliar eyes. A few are wrong.

An appraisal of this book is difficult. The reviewer imagined that the author would assume a fair knowledge of general biochemistry on the part of the reader, and would emphasize the unique chemistry of the brain in this special treatise. Instead, Page takes the burden of reviewing a good many parts of biochemistry as he might have done for a semi-advanced, general treatise. One wonders why he did this. It appears that he felt a neglect of general biochemistry by students of the brain, as well as neglect of the brain by students of general biochemistry. Judged under this emotion the book may be said to contain valuable information having considerable range of pertinence to the needs of two classes of people—negligent biochemists and negligent clinicians. Some of the chapters give succinct reviews of what is known, and desirable emphasis upon what is known only so well that fields for discovery can be named.

Nevertheless, to judge the book only on this basis would be to remain merely a partner to an indictment. It would have been better had neglectful biochemists and neglectful clinicians been left in the nether regions to which they have assigned themselves. The author might then have set forth upon a reorientation of his subject with an analysis of its major topics and issues. A book may be written the better if a need is in mind but the need must be met by intellectual analysis. In this case the need should not have precluded focusing the lights of contingent subjects; the difficulty is that too often one has to hunt for the foci.

To illustrate the fact that general topics have pushed aside much that might

have been expected, one need only call attention to a few items. On page 338 is the following remarkable statement. "Centers in the brain that are believed to control, or at least partially to regulate, metabolism have been the subject of extensive investigation. *This problem cannot be discussed adequately within the limits of this book.*" Arbitrary limitation of scope is permissible and often advisable but the reviewer must italicize this statement because Page's book ostensibly concerns what Mathews called "the master tissue." Is it *a* tissue? Rather is it not a set of many tissues? Too often the older chemists made a *Brei* of the whole thing. It is no longer satisfying to read of increments of this or that component of the whole. One wants to know where the increment went. Page gives too little aid to the biochemist who needs a wider knowledge of morphology and no inspiration to the morphologist who should know the opportunities of histochemistry. Quite properly the topics are not limited to what is found within the brain cavity and yet the index contains but one reference to the carotid sinus. The exciting story of acetyl choline is obscured by too perfunctory an account of the main findings.

One might allow the author his indulgence in the last chapter, *The Brain and Thought*, were it not that it may undo what his assembly of factual material could accomplish. For all its naïveté science manages to force conviction by resort to its experimental method, and while the scientist confines his attention to his proper data he can write a book of some usefulness even if it has little logical order and no persuasiveness. There was room enough to have retained the bold yet simple outlook, but Page steps far enough outside the confines and might be put upon by professional philosophers. One can imagine them noting the carry-over of undesirable connotations in words relating to philosophical and theological ideas that are irrelevant to the immediate subject. It is a far cry from doctrinal discussions of epistomology to the simple fact that this book presented certain confusions of thought that worried the reviewer into a state of physical excitement. While the psychiatrist has infinitely more complex matters to consider I should think that he and the chemist sympathetically could begin a modest coöperation within the general category of facts of which this experience of the reviewer is a trifling example. With charity for those who will enjoy the book's last chapter I feel it to be an indulgence, the privilege of which Page failed to win.

W. M. C.

Fifty Years a Country Doctor. By WILLIAM N. MACARTNEY. 584 pp. $3.50. (*E. P. Dutton & Co., New York, 1938.*)

After a vivid account of life on the house-staff of a large New York hospital a half century ago, the author gives a systematic record of his management of various problems that confront the physician and surgeon in general practice. Among the problems to be faced, grappled with, and solved to the best of his ability, we find all manner of disease, of humanity, and of physical obstacles. The author is no therapeutic nihilist; he discovered early that it was his duty to make the best use of whatever implements nature and man's invention had put

at his disposal, specific cure or no specific cure. No one could agree with all his conclusions, for they are highly personal and individualistic; the writer, like all good general practitioners, is individualistic, independent, self-confident, and not above a polemic.

The writer is, apparently, no novice in the use of English; errors in this department are negligible. He both "practices" and "practises" his profession; as his work was done both in Northern New York and Canada perhaps he is purist enough to "practice" in this country and "practise" in Britain. To the reviewer the interest of the book lies, not so much in the medical information it contains, as in the character and personality of the highly intelligent physician which it portrays. He wants no help and asks no quarter. He is bored with the paternalism of the time. Those who feel sorry for the general practitioner give the writer a "cervicodynia"; the general practitioner, he intimates, is having the time of his life.

J. T. K.

The Patient and the Weather. By WILLIAM F. PETERSEN, M.D. with one chapter by ALEXANDER J. NEDZEL, M.D. Vol. IV Organic Disease, Part 1, 663 pp.; Part 2, 729 pp.; Part 3, 651 pp. Illus. $10.00 or $11.00 per part. (*Edwards Brothers, Inc., Ann Arbor, Mich., 1937–38.*)

In this volume Petersen presents his ideas of the rôle of the meteorological environment in specific diseases. His theory is that each polar air mass with its low temperature and high barometric pressure produces in the body an increase in blood pressure, smooth muscle contraction, anoxia, and relative alkalosis (ARS phase = Anoxia and Anabolism, Reduction and Spasm) which is followed by a reverse phase (COD phase = Catabolism, Oxidation, Dilatation). Though normally without pathological significance these phase alterations when exaggerated in magnitude or frequency may initiate disease under certain circumstances (hereditary background, age, sex, infection, mental stress, etc. are conditioning factors) in the unstable individual and will profoundly affect the clinical course of diseases. Evidence in support of this view is derived from correlating the daily meteorological changes with the onset and changes in symptoms in individual clinical cases selected from medical journals, hospital records and from the author's personal experience. Elaborate meteorgraphs are given upon which the clinical incidents are marked. As less reliable but still supporting evidence the author presents the geographical and seasonal distribution of mortality for the disease.

The author applies his theory in Part I to the cardio-vascular-renal diseases; in Part II to endocrine disturbances (thyroid and diabetes), blood dyscrasis and tuberculosis; and in Part III to a wide variety of surgical problems such as gastric ulcer, cholecystitis, appendicitis, ectopic pregnancy, glaucoma, etc. For each condition Petersen has included a review of the literature with extensive quotations.

One example will suffice to illustrate the type of argument and evidence. With

regard to endocarditis Petersen believes that the changes in blood pressure associated with the alternating ARS and COD phases result in a changing mechanical impact at the valvular margins. This leads to greater adhesiveness of the valve membrane and thus to bacterial localization on the valves. The environment further affects the disease since the COD phase is associated with a greater permeability of the membranes to bacteria. The evidence presented to support this is 1) selected clinical cases showing a relationship of onset, symptoms and death to meteorological changes, 2) mortality data showing bacterial endocarditis primarily as a disease of the northern storm-track latitudes, 3) experiments on dogs (chapter by Dr. Nedzel) showing microscopic changes in the endothelium of the valves following an increase in blood pressure produced by injections of pitressin and evidences of valvular endocarditis with subsequent bacterial injections.

There can be no doubt that changes in the environment may exert a very definite effect on the physiological activity of the body and the production and clinical course of diseases but proof of this is difficult to obtain. Unfortunately these three parts of Volume IV yield no evidence which will stand scientific criticism. The extensive review of clinical cases is unconvincing; the graphs are complex and difficult to interpret; there is no statistical treatment of material; and very few experimental data are cited. There is a lack of clarity of thought and presentation, and much repetition. The value of these books lies in the fact that they focus attention on a problem which has been largely neglected, chiefly because of the multiplicity of factors involved.

A. M. B.

Biochemistry for Medical, Dental and College Students. By BENJAMIN HARROW. 383 pp. Illus. $3.75. (*W. B. Saunders Co., Philadelphia and London, 1938.*)

The reviewer, noting the slender size of this book (383 pages) and the variety of its topics (24 chapters), guessed that we have another text written as a long series of brief assertions. Perusal confirmed the guess.

At this level of exposition the usual topics will be found well condensed and brought fairly well up to date.

Boswell reports Dr. Johnson to have remarked that lectures are superfluous now that so many books are available. One wonders whether there may not be use of lectures still, if only to emphasize evidence in terms of which students can profitably remember some parts of this field of knowledge. It has become so vast as to force text-book writers to the style of the abstractor.

W. M. C.

The Technique of Contraception. By ERIC M. MATSNER. 4th ed. 50 pp. (*National Medical Council on Birth Control, New York, 1938.*)

This small pamphlet should not be overlooked because of its size, as it is

intended to be only an outline of contraceptive technique and not a comprehensive discussion of the subject of contraception. It fills an important place in the available medical literature concerning this problem.

Dr. Matsner, a specialist in this field, is unusually well equipped to write such a monograph. In a clear, concise and well organized manner he gives first an excellent account of the indications for contraceptive advice, then of the various methods of contraception, those considered dependable and harmless as well as those considered undependable and harmful. He discusses methods from the point of view of their reliability. He also mentions methods which are under experimental study. The pamphlet is well illustrated and has an adequate bibliography.

In the foreword Dr. Frederick C. Holden says, "The keynote of modern medicine is prevention. Physicians realize that as a preventive measure the control of human fertility is not only rational but essential in any progressive program of individual and public health. . . . With the recent recognition of contraception as an integral part of medical practice by the American Medical Association, the provision of competent contraceptive instruction is a necessary part not only of gynecological and maternity services but of the general public health program."

Owing to the fact that the technique of contraception has been taught in medical schools only in very recent years, many practicing physicians have a most inadequate knowledge of the problem. For them as well as for medical students this outline will prove of immense value in furnishing a summary of authoritative present day opinion regarding methods.

B. L. M.

Outline of Roentgen Diagnosis. By LEO G. RIGLER. Atlas edition, 212 pp. + 254 illus. $6.50. (*J. B. Lippincott Co., Philadelphia, 1938.*)

I cannot compliment this work too highly. It is a syllabus and should make roentgenologic teaching much simpler. The Atlas embodies excellent illustrations of the most common types of diseases as revealed by the x-ray. It is a reference book that should be in every radiologist's library, and should serve as an excellent example for teachers to follow. It is not likely that a book of this character will overlap with other books on x-ray diagnosis. It should have a place of its own.

C. A. W.

Anus, Rectum, Sigmoid Colon; Diagnosis and Treatment. By HARRY ELLICOTT BACON. 855 pp. Illus. $8.50. (*J. B. Lippincott Co., Philadelphia, 1938.*)

Within the past two decades proctology has grown and has attained its place among the other specialties. Until the past few years there have been few text-books confined to the subject of the lower intestinal tract. Only as far back as 1828 Frederick Salmon published, in London, the first text-book upon diseases of the rectum, "Practical Treatise on Stricture of the Rectum." Recently numerous first-class text-books dealing exclusively with diseases and conditions

of the rectum and colon have appeared and the literature on these subjects now rivals that of the other specialties. Dr. Bacon's book is the most recent addition to demand a place on our bookshelves.

This work is well planned, well written and copiously illustrated with charts, drawings, most of them original, and many photomicrographs. There are splendid résumés of lymphopathia venereum, and anal and rectal stricture; and there is an excellent chapter on anal fistulae. In his handling of the malignant tumors of the anus and rectum the author covers all the accepted foreign and American surgical literature in this field and discusses, in some detail, 17 operative procedures.

Owing to the author's encyclopedic method of covering the various topics, I feel that this work is not concise enough to serve as a medical students' text-book but, for the same reason, it is more valuable to the general surgeon contemplating surgery in this field, and to the proctologist or specialist. Full and comprehensive bibliographies at the end of each chapter are extremely valuable.

Dr. Bacon is to be congratulated and sincerely thanked for modernizing this large field. His work will be appreciated for years to come.

T. R. C.

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John Whitridge Williams: Academic Aspects and Bibliography. By J. Morris Slemons. Foreword by John C. French. 1935. 121 pages, frontispiece, 12mo, boards, $1.50.

The life of John Whitridge Williams, as Dr. Slemons has told the story, is an essential chapter in the history of American medicine. Dr. Williams shared largely in the far-reaching effect The Johns Hopkins School of Medicine has had on instruction and research and as a brilliant investigator in his own specialty and the author of an authoritative textbook, he advanced scientific knowledge and human welfare. Dr. Slemons had been aware of the human interest of his subject; and by virtue first of intimate association as student and junior colleague and later as scientist and practitioner in the same field thousands of miles away, he has been able to bring to it both warm personal feeling and scholarly detachment. He has performed a congenial task with distinct success.

Papers and Addresses. By William Henry Welch. 1921. Three volumes, 2013 pages, 4to, $20.00.

Dr. Welch's writings are scattered through a great variety of publications, many of which are more or less inaccessible. It was accordingly decided to publish his numerous papers and addresses. These have been collated under the following headings: Volume I. Pathology, Preventive Medicine. Volume II. Bacteriology. Volume III. Medical Education, History, Miscellaneous, Bibliography, Index.

Surgical Papers of William Stewart Halsted. 1928. Two volumes, 1232 pages, illustrated, 4to, $15.00.

When one considers the number, originality and importance of Dr. Halsted's contributions to surgery and their scattered and often not readily accessible form in various journals and reports, it will be realized that the publication of his collected papers not only constitutes a worthy tribute to his memory, but also renders a welcome and much needed service in making available these significant contributions.

William Stewart Halsted: Surgeon. By W. G. MacCallum. 260 pages, 18 illustrations, 8vo, $2.75.

A brief history of the life of the first surgeon of The Johns Hopkins Hospital. Dr. Halsted, who died in 1922, was one of the four men whose names are best known in the establishment of the fame of The Johns Hopkins Medical School and Hospital. He formed a new school of surgery and trained in his own methods and modes of thought many of the men who are now prominent surgeons in this country.

Franklin Paine Mall: The Story of a Mind. By Florence Rena Sabin. 355 pages, 8 illustrations, 8vo, $2.75.

This book seeks to give the story of the development of Mall's mind and its influence on medical science and on medical education. The writer worked for twenty years under Mall and the theory of education outlined in this volume was thus learned directly from him. It is hoped that the new generation of medical students who have never known Mall may gain, through these pages, some idea of how this man has influenced their lives and that, too, they may gain an insight into the difficulties through which the generation that preceded theirs won the privileges they now enjoy. Mall is but little known to the public, but one has only to mention his name to any of his colleagues to sense that he was one of the leaders of his generation.